

**REPORT TO THE SENATE ARMED SERVICES  
COMMITTEE AND THE HOUSE OF REPRESENTATIVES  
NATIONAL SECURITY COMMITTEE**

**on**

**Department of Defense  
Animal Care and Use Programs 1994**

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## List of Acronyms

AAALAC	American Association for Accreditation of Laboratory Animal Care
AALAS	American Association of Laboratory Animal Science
ACLAM	American College of Laboratory Animal Medicine
APHIS	Animal Plant Health Inspection Service
ASBREM	Armed Services Biomedical Research Evaluation and Management
ASD(HA)	Assistant Secretary of Defense (Health Affairs)
ASTMP	Army Science and Technology Master Plan
AWA	Animal Welfare Act
CRISP	Computer Retrieval Information of Scientific Projects
DDR&E	Director, Defense Research and Engineering
DoD	Department of Defense
DTIC	Defense Technical Information Center
ELISA	Enzyme Linked Immunosorbent Assay
FEDRIP	Federal Research in Progress
FDA	Food and Drug Administration
FY	Fiscal Year
HASC	House Armed Services Committee
IACUC	Institutional Animal Care and Use Committee
IG	Inspector General
ILAR	Institute of Laboratory Animal Resources
JDL	Joint Directors of Laboratories
JTCG	Joint Technology Coordinating Groups
LAM	Laboratory Animal Medicine
NIEHS	National Institute of Environmental Health Sciences
NIH	National Institutes of Health
NTP	National Toxicology Program
OIG-DoD	Office of the Inspector General, Department of Defense
OPRR	Office for the Protection from Research Risks
OSD	Office of the Secretary of Defense
PADPRP	Poly (ADP-ribose) Polymerase
PCR	Polymerase Chain Reaction
RDT&E	Research, Development, Test, and Evaluation
S&T	Science and Technology
SEB	Staphylococcus Enterotoxin B
STO	Science and Technology Objective
TAPSTEM	Training and Personnel Systems Science and Technology Evaluation and Management
USAMRMC	U.S. Army Medical Research and Materiel Command
USDA	United States Department of Agriculture
WRAIR	Walter Reed Army Institute of Research

**Section****INTRODUCTION/OVERVIEW**

The National Defense Authorization Act for Fiscal Year 1995, Report of the Committee on Armed Services, House of Representatives, in H.R. 4301, 103rd Congress, Second Session, HASC Report No. 103-499, "Animal Research," requested the Secretary of Defense to "develop a mechanism for providing Congress and interested constituents with timely information about its animal use programs, projects, and activities, both intramural and extramural." Therefore, this report provides a detailed accounting of animal use and describes the development of a database that will be accessible to the public. In addition, the report requested actions to enhance oversight and public access to information regarding DoD uses of laboratory animals (see I.3, Scope of Report).

This report describes the DoD response to these requests and also updates and expands information on animal cost and use programs contained in the 1993 report to Congress. The report covers animal research conducted by the DoD including education, training, and testing both in DoD laboratories and by extramural projects funded by the Department for fiscal year (FY) 1994. This report does not include information on animals used by the DoD solely for the purpose of food preparation for human or animal consumption, ceremonial activities, recreation, or the training, care, and use of military working animals.

**I.1 REQUIREMENTS FOR USE OF ANIMALS IN THE DoD**

The continued use of animals by the DoD in research, education, and training is absolutely essential to ensure sustained technological superiority of the U.S. in military operations in defense of our national interests. The DoD's animal use programs ultimately translate into maintaining and improving military readiness as well as reduction in morbidity and mortality associated with military operations. They contribute directly to ensuring that deployed service men and women may accommodate to the hazards associated with military operations. Additionally, humanitarian benefits of the DoD investment in animal research are shared on an international basis to improve the quality of life of both humans and animals.

Although many alternatives to animal use have been discovered and applied by the Department, there remain situations in which there are no acceptable alternatives. While fundamental scientific and biomedical principles have been explored and understood using non-living and cell culture models, the complex interactions within the human (e.g., organ, endocrine, circulatory and related systems) and with the environment have not been effectively modeled for all areas of concern to the DoD. For example, disease has been and remains a major cause of death and disability in military conflicts. During Operations Desert Storm and

Restore Hope, outbreaks of respiratory diseases, shigellosis and other diarrheal diseases, leishmaniasis and other parasitic diseases including malaria continued to threaten the health and well being of our troops.

As a consequence, DoD must develop the materiel and technological means to best protect and sustain the health and well being of service men and women despite battle and disease-non-battle threats, and to provide the best medical treatment possible to those who become casualties. This responsibility underlies the need for the DoD to conduct research, and to train and educate military health-care providers in the most effective medical management of battlefield casualties. Unlike medical counterparts found in civilian emergency medicine and trauma management, battlefield health care must very often be provided in an austere, harsh and hostile environment hours away from a definitive care hospital. Whereas an urban gunshot patient in a modern civilian shock and trauma center will be supported and resuscitated by a full complement of medical staff with a plentiful supply of oxygen, fluids, medications, surgical intervention and nursing, the combat casualty may be supported only by a single aidman and the medical supplies he can carry.

Another visible area requiring the DoD to use animals in research is the need to develop vaccines and drugs to protect, sustain and treat service men and women during military operations. Similar to health-care delivery, these research programs are focused on the disease-causing threats most important to the military missions. Ethical concerns, as well as the regulatory requirements of the Food and Drug Administration (FDA), necessitate that candidate vaccines and drugs be demonstrated to be safe in laboratory animal models prior to initiation of human studies. The statutory basis for such ethical concerns and FDA regulations is the legitimate matter of ensuring human protection from dangerous and ineffective treatments. Indeed, during the final stages of vaccine and drug development, large-scale testing is conducted using human subjects, often individuals who are naturally exposed to the disease in question.

## **I.2 DoD POLICY GOVERNING ANIMAL RESEARCH**

While essential to the protection of military personnel, animal research is considered a trust. DoD agencies have consistently adhered to direction (DoD Directive 3216.1, "The Use of Animals in DoD Programs", 1995) (Appendix A) to follow the federal regulations that govern the use of animals in order to prevent unnecessary suffering and to minimize the numbers of animals used. This most recent revision to DoD Directive 3216.1 (1995) strengthens the requirements for nonaffiliated membership on Institutional Animal Care and Use Committees (IACUCs) and directs all DoD animal use facilities that maintain animals for research, testing and training to apply for American Association for Accreditation of Laboratory Animal Care (AAALAC) accreditation. DoD Policy Memorandum entitled "Department of Defense (DoD) Policy for Compliance with Federal Regulations and DoD Directives for the Care and Use of Laboratory Animals in DoD-

Sponsored Programs"<sup>1</sup> (Appendix B) specifies training requirements for nonaffiliated IACUC members and implements a standard format for animal use protocols (Appendix C), a standard checklist for IACUC inspections (Appendix D), and a standard reporting requirement for all animal use research to support a publicly accessible database. All animal research must conform to requirements of the 1966 Animal Welfare Act (P.L. 89-544) as amended in 1976 (P.L. 94-279) and 1985 (P.L. 99-198), as well as the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*, (fifth edition, 1985, NIH86-23) and the requirements of the applicable regulations of the United States Department of Agriculture (USDA). Although the Animal Welfare Act does not apply to mice of the genus *Mus* and rats of the genus *Rattus*, the DoD voluntarily conducts research with these exempt species with the same procedures defined in the Animal Welfare Act for other mammals. At the same time, DoD biomedical researchers have aggressively developed novel procedures to replace, reduce, and refine the use of animal subjects during experimentation.

### I.3 SCOPE OF REPORT

The report of The Committee on Armed Services, House of Representatives (HASC Report No. 103-499) specifically requested the following actions to improve DoD-sponsored animal research:

- Develop a mechanism for providing Congress and interested constituents with timely information....about animal use programs that includes information on research goals and justifications, costs, procedures, kinds and numbers of animals used, and pain evaluation;
- Submit a report to the House Armed Services Committee (HASC) on the development and implementation of the mechanism to provide animal use information;
- Ensure that the Federal Research in Progress (FEDRIP) database searches become a prerequisite for approval of new research projects involving animal use;
- Report to the HASC on plans to improve community representation on DoD research facility IACUCs, and appoint animal advocates as the bona fide community members to the IACUC at each DoD facility; and
- Establish aggressive programs to replace, reduce, and refine current uses of animals.

In addition, the National Defense Authorization Act for Fiscal Year 1995 Conference Report, section 2182, 103-701 "urged the DoD to seek accreditation of all DoD animal facilities as expeditiously as possible."

This report will address each of these requests and provides information on DoD animal care and use for FY94. This report includes: description of plans to

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<sup>1</sup>References to the DoD Policy Letter will be cited in the text as DoD 1995 Policy Letter.

implement a publicly accessible database on animal use programs in the DoD (Section II); information on oversight of DoD animal care and use programs (Section III); information on accreditation of DoD laboratories by the AAALAC (Section IV); Service and DoD animal use and cost profiles by research categories (Section V); DoD initiatives to promote alternative methods that replace, reduce, or refine animal use (Section VI); glossary (Section VII); and a list of references in order of citation (Section VIII). Several appendices are included that provide more detailed information to support these sections.

### **I.3.1 Publicly Accessible Information on Animal Use in the DoD**

As requested by the HASC, the Department is implementing a publicly accessible database analogous to the National Institutes of Health (NIH) Computer Retrieval Information of Scientific Projects (CRISP) System. The CRISP System is a biomedical database containing information on research projects supported by the United States Public Health Service, as well as information on intramural research programs of the NIH and FDA.

Currently, research, development, test and evaluation (RDT&E) efforts represent a large percentage of DoD animal use included in a work unit summaries database maintained by the Defense Technical Information Center (DTIC). The DoD has recently directed that animal research performed or sponsored by DoD intramural laboratories and extramural contracts, as well as that research primarily done at Clinical Investigation Services associated with the major medical centers be entered into DTIC. These data provided to DTIC are analogous to the information in the NIH CRISP System, and will form the basis for this new DoD database. The DoD will make FY94 information on animal use in research, testing, education and training available to the public this year. The database will be accessible to the public through FEDRIP and/or Internet by October 1, 1995. More detailed information is presented in Section II.

### **I.3.2 Oversight of DoD Animal Care and Use Programs**

DoD animal use oversight is reviewed in Section III. In general, internal and external oversight provisions for animal research conducted by the DoD are as stringent as those for research in any other department of the Federal Government. As a matter of policy, the DoD abides by the applicable federal regulations pertaining to animal care and use, including provisions for oversight. All DoD facilities and extramural institutions sponsored by the DoD must submit proposed protocols of animal use to an IACUC. DoD-operated animal use sites engage an IACUC to review proposed animal protocols to ensure compliance with the Animal Welfare Act. The goal of the IACUCs is to ensure compliance with requirements for animal welfare and address concerns of the community. The revised DoD Directive 3216.1 (1995) continues to specify that DoD IACUCs shall conform to the provisions of the Animal Welfare Act. Each IACUC serves as an independent decision-making body of the institution and establishes policy for the care and use of animals at that facility in accordance with applicable DoD directives, federal law and regulations.

Central to this oversight process is the review of all proposed animal use research protocols. The DoD Inspector General's *Review of the Use of Animals in Department of Defense Medical Research Facilities* (February 1994) recommended that the DoD develop a standardized research protocol request form. The DoD 1995 Policy Letter has established a DoD Standard IACUC Protocol Format that is included in Appendix C. In accordance with HASC recommendations, the standard format requires that the FEDRIP databases be searched to prevent duplication of ongoing federally funded research. The protocol must justify the use of animals, including consideration of alternatives, justify the choice of species and the number of subjects, and include a literature search and assurance that the work does not needlessly duplicate prior experimentation. The protocol must specify procedures to be used with animals, methods to avoid or minimize pain (including a literature search for possible alternatives), qualifications of persons conducting procedures with animals, and disposition of animals at the termination of the work. The IACUC ensures that personnel involved in animal-based studies are properly trained and, if necessary, establishes a training program to support the staff. The IACUC inspects facilities and animal care programs at least twice annually and prepares a written report, including a plan to address all significant deficiencies. The IACUC enforces compliance with the procedures specified in the protocols by conducting inspections, evaluating and if necessary investigating reports of deviation from approved procedures. Finally, the IACUC serves as an impartial investigator of reports of violations of good animal practices and is empowered to suspend the use of animals for protocols not conducted in accordance with Animal Welfare Act or institutional policy.

The revised DoD Directive 3216.1 (1995) clarifies the composition, membership, and training requirements of the IACUC. The changes address the HASC request to improve community representation and to appoint animal advocates to DoD IACUCs, consistent with a recommendation of the Inspector General Report of February 1994. The revised Directive (1995) increases the minimum membership of all DoD IACUCs from three to five. In addition, it specifies that

"there shall be at least one non-scientific member on the IACUC. In addition, there shall be at least one member representing the general community interest who is nonaffiliated with the research facility. The nonaffiliated member and the non-scientific membership can be filled by the same person. To ensure community representation at each meeting and inspection, an alternate to the nonaffiliated member shall be designated for all IACUCs having a single nonaffiliated membership."

This Directive exceeds the requirements of the Animal Welfare Act and is further strengthened by the DoD 1995 Policy Letter which requires a minimum of eight hours of training for nonaffiliated members. All IACUC's must strive toward implementation of these requirements by October 1, 1995.



The IACUC of each facility performs semiannual program reviews of all animal use areas. The DoD 1995 Policy Letter strengthens that process by establishing a standardized semiannual review checklist that outlines the areas required for IACUC review. This guidance is consistent with the recommendations of the DoD IG report of February 1994 (Appendix E). A formal report of inspection shall be prepared twice annually, noting the use of the checklist, and indicating all major and minor deficiencies, a plan for correction of all major deficiencies, signatures of IACUC members conducting the inspection, and a statement indicating whether there are or are not minority opinions.

Responsibility for oversight of the Department's science and technology programs rests with the Director, Defense Research and Engineering (DDR&E). Her staff, in conjunction with representatives from the Services, annually review the science and technology efforts to ensure they are fully coordinated and without unnecessary duplication of effort. The preponderance of animal use within the Department occurs in biomedical programs. These activities receive specific oversight from the Armed Services Biomedical Research Evaluation and Management (ASBREM) Committee, which was created by congressional direction in 1981. The ASBREM is chaired by the DDR&E and co-chaired by the Assistant Secretary of Defense (Health Affairs). The overall biomedical effort is carefully integrated and reviewed to eliminate unjustified duplication of effort by seven subordinate Joint Technology Coordinating Groups reporting to the co-chairpersons.

### **I.3.3 Accreditation of DoD Laboratories by AAALAC**

AAALAC accreditation of DoD animal use programs is reviewed in Section IV. In response to the National Defense Authorization Act for Fiscal Year 1995, Conference Report (2182, 103-701), DoD Directive 3216.1 (1995) states that all DoD laboratories that maintain animals for use in research, testing or training shall apply for AAALAC accreditation. Currently, of the 34 separate DoD animal facilities in the U.S., 27 or 79% already have received accreditation, a record that exceeds the 41% accreditation rate for civilian research laboratories registered with the USDA. Other DoD facilities in the U.S. and overseas have either applied for accreditation or are in the process of preparing an application for accreditation. Animal use programs in the DoD strive to meet all the requirements of AAALAC. AAALAC's philosophy of accreditation is steadily evolving from an emphasis on physical facilities (engineering standards) to a more comprehensive evaluation of the total laboratory animal care and use program (performance standards). Consequently, research units that were previously regarded as unaccreditable until major facility renovations or upgrades were completed are now actively pursuing AAALAC accreditation. By DoD directive, all animal use facilities shall apply for AAALAC accreditation and, therefore, will be site visited by them. The Inspector General's *Review of the Use of Animals in the Department of Defense Medical Research Facilities* confirmed the effectiveness of animal husbandry programs in DoD facilities and concluded that although not all facilities were AAALAC accredited, animals in DoD facilities were maintained in healthy environments and treated humanely. As stated in the report, "The inspection teams were completely satisfied

with the health and welfare of the animals in DoD research facilities. ... All the personnel assigned the care of the animals were competent, interested, and committed to the humane care of the animals."

The DoD is committed to accrediting its research programs. In fact, of the two programs identified by the DoD IG in their 1994 report as "not substantially in compliance" with DoD regulations and the Animal Welfare Act, both are now fully accredited by AAALAC. In recognition, the conference report on the FY95 National Defense Authorization Act stated that "the conferees applaud the Department's expeditious efforts to gain accreditation for these facilities."

#### I.3.4 DoD Animal Use and Cost Profiles by Research Category

A profile of DoD animal use and costs is provided in Section V. In this report, we have adopted a more detailed system for classifying animal use that includes eight categories with twenty-three sub-categories: eight medical research, four non-medical research, three clinical research, two training, and six other categories of studies and use. Detailed charts and graphs are included in Section V.

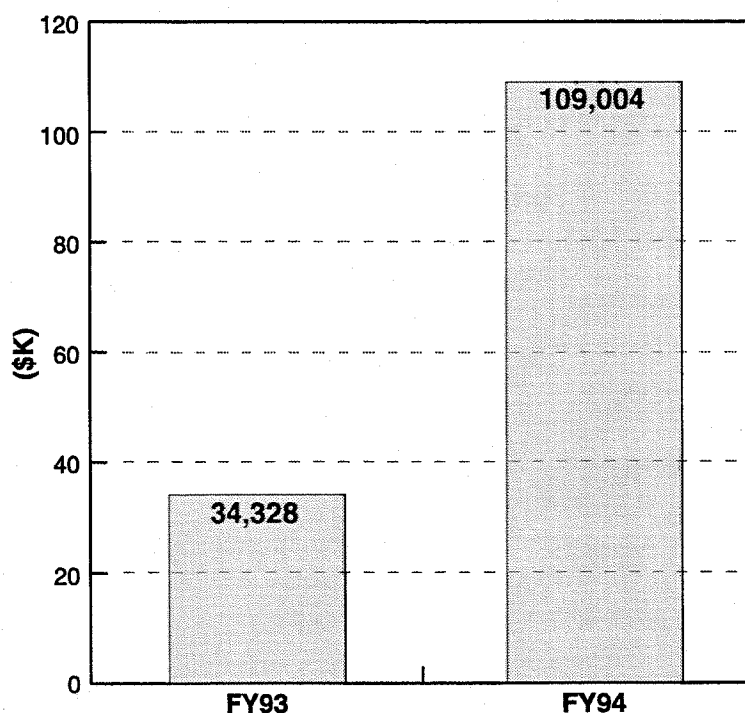


Figure I-1. Use of Fish in FY93 and FY94

During 1994, the cost of animal-based research was approximately \$146 million. In 1994, total animal use increased by 8%, which is largely a result of implementing alternatives to animal research. The increase in the use of fish rather than other animals higher on the phylogenetic scale was the largest contributor to the increase in animal use in FY94 (Figure I-1). The majority of these fish replaced other species

higher on the phylogenetic scale. Table I-1 summarizes the major animal use statistics for DoD research. In addition, it should be noted that no animals were used for development or testing of offensive weapons.

### I.3.5 DoD Initiatives to Promote Alternative Methods that Replace, Reduce, and Refine the Use of Animals

Congress requested that the DoD establish aggressive programs to replace, reduce, and refine current uses of animals. Over 130 examples of DoD efforts to replace, reduce, and refine the use of animals in research are reviewed in Section VI. Animal research is an essential part of the scientific process, but it is always undertaken after due consideration of alternatives. The DoD 1995 Policy Letter directs the use of a DoD Standard Protocol Format that specifically requires each protocol to consider alternatives to the use of animals and to justify the animal model selected. In addition, all protocols that involve unrelieved pain or discomfort require consultation with a veterinarian, and a specific database search for scientifically acceptable alternatives to the proposed method. Each protocol that involves animals in research or training must explain the need for the animal research and defend the choice of species as the most scientifically valid model. Often, economies of time and resources are gained when scientifically valid alternatives to animal use are available. Our review of current animal research reveals that scientists in the DoD have developed or adopted many alternative methods based on ethical considerations and other inherent benefits. One DoD organization, the U.S. Army Medical Research and Materiel Command has established a major objective to develop replacement, reduction, and refinement strategies for the use of animals in research. In FY94, approximately \$2 million was invested in this objective. In addition, the Department sponsors conferences and workshops to promote alternatives to animal research. The DoD sponsors a five-year grant with the Institute of Laboratory Animal Resources of the National Research Council to develop institutional training materials, education, and publications in support of DoD laboratory animal

**Table I-1. Summary of DoD Animal Use Statistics**

Total Animal Use by Species	% of Total
Rodents, fish, amphibians and birds	98
Rabbits	0.5
Farm animals (i.e., sheep, pigs, cows, horses)	1
Dogs, cats, nonhuman primates, marine mammals	0.6
Other	<0.1

Percentages may not add up to 100% due to rounding of calculations.

Total Animal Use by Category	% of Use	% of Cost
Medical RDT&E	76.8	70
Non-Medical RDT&E	10.9	16
Clinical Investigation	3.4	9
Adjuncts/Alternatives	8.6	1
Training & Instructional	1.1	2
Breeding Stock	<1	<1
Classified Secret or Above	<1	<1
Offensive Weapons Development	0	0

Percentages may not add up to 100% due to rounding of calculations.

care and use programs. The Institutional Animal Care and Use Committee process also includes a strong emphasis on consideration of alternatives in all new protocols. Table I-2 describes several examples of new initiatives that replace, reduce and refine the use of animals.

In conclusion, it is the policy of the DoD that animal utilization will be conducted in full compliance with the Animal Welfare Act and that animals are used in research only when scientifically acceptable alternatives are not available. At the same time, the use of animals in research is essential to protect the health and lives of servicepersons, and the DoD will be engaged in biomedical research that involves the use of animals for the foreseeable future.

**Table I-2. Examples of DoD Initiatives for Replacement, Reduction, and Refinement of the Animals Used in Research**

Development of fish (rainbow trout, zebra danjo fish and medaka) as predictive models for epigenetic carcinogens has reduced mammalian animal use in carcinogenesis studies.

Development of a computer model for predicting the transfer of toxic chemicals across the intestinal mucosa and into the blood stream.

Cell cultures are being evaluated to replace mice as a host assay for detecting and identifying anthropol-borne viruses.

Cell and organ cultures to replace the rat in regulated mucin gene expression in airway injury studies.

A contract with the Cooperative Human Tissue Network provides human skin biopsies that replace the use of hairless guinea pigs and weanling domestic swine.

Rats and swine may replace cynomolgus monkeys as an alternative model for hepatitis E.

Ocular researchers are using eyes purchased from local cattle processing plants for studies instead of live rabbits.

Development and validation of fish immune responses as a biomarker to replace laboratory mammals.

Development and use of amphibian models (*Xenopus laevis* - frog) for assessing teratogenesis assays significantly reduce mammalian animal use .

Training of professionals by interactive videos and innovative teaching techniques, e.g., laparoscopic instruments on synthetic sponges, reduces the use of animals.

Comparison of *in vitro* results using tissues derived from the same animal to help validate the *in vitro* assay as an alternative to live animal use in toxicology research.

**PUBLICLY ACCESSIBLE  
INFORMATION ON  
ANIMAL USE IN THE DoD**

**Section**



HASC Report 4301 (1995) requested the Secretary of Defense to "develop a mechanism for providing Congress and interested constituents with timely information...about [DoD] animal use programs, projects and activities, both intramural and extramural." In response to this request, the Department is implementing a publicly accessible database analogous to the National Institutes of Health (NIH) Computer Retrieval of Information of Scientific Projects (CRISP) System. The CRISP System is a biomedical database containing information on research projects supported by the United States Public Health Service, as well as information on intramural research programs of NIH and the Food and Drug Administration. The animal use database is to be accessible to the public through Federal Research in Progress (FEDRIP) and/or Internet by October 1, 1995. FEDRIP is a database that provides access to information about ongoing federally funded research projects in the physical sciences, engineering, and life sciences. It is produced and administered by the National Technical Information Service, and includes contributions from federal agencies such as the National Aeronautics and Space Administration, NIH, the National Science Foundation, the U.S. Department of Agriculture, the National Institute for Occupational Safety and Health, and the Department of Energy, among others. Internet is a global computer network and a rapidly growing forum for information access and exchange worldwide.

A publicly accessible database is to be developed from the current work unit summary system of the Defense Technical Information Center (DTIC). DoD organizations performing Defense research, development, test & evaluation (RDT&E) projects are currently mandated to provide annual reports of research to the DTIC. The DTIC maintains these work unit summaries in a database. While the majority of DoD animal use occurs in RDT&E projects, some is performed in clinical investigations programs previously not mandated to provide work unit summaries. The DoD has directed that summaries of these non-RDT&E DoD animal research projects also be entered into the DTIC database.

The data in the DTIC system are analogous to information in the CRISP system. Similar data include: Title (of research work unit), Point of Contact at Laboratory (or Principal Investigator), Performing Organization Activity Name, Fiscal Year, Funding, Approach and Objective of the research, and indexing terms/descriptors/keywords. These data will serve as the basis for information in the publicly accessible database.

Military activities that house, care, or use animals have provided a work unit summary for any animal-based research. A work unit summary may refer to a single protocol or a series of protocols that are performed in a given category of

animal use. These data from work unit summaries were extracted, revised as necessary to meet proprietary or classified information concerns prior to public release, and placed in the publicly accessible database.

The timeline for the entire public access effort is as follows:

1. Develop the capability to collect data for all DoD ongoing animal-based research projects. This involves extraction of data from work unit summaries in the DTIC database and creation of a data collection tool for projects not currently mandated to report to DTIC. These efforts were completed by January 31, 1995. The collected data include the following information:

- **Accession Number:** Identification number to be given by the database.
- **POC/Author:** Primary contact for the work unit. This can be the Public Affairs Office, Principal Investigator, Department Chief, Clinical Investigation Service Director, etc.
- **Title:** Title of the work unit.
- **Funding Fiscal Year:** This will be FY94 for all work units in this database. Subsequently, it will be the current fiscal year of the reporting requirement.
- **Funding:** This is the proposed funding for the entire work unit for a given fiscal year. The funding will include civilian salaries, cost of animals, cost of materials, etc. - all costs related to the work unit except military salaries.
- **Performing Organization:** The name of the activity where the work unit is performed.
- **Objective and Approach:** This section will be a narrative on the objectives and the approach of the work unit. This narrative will provide a general summary of the work unit.
- **Indexing Terms (Descriptors):** A list of indexing terms or keywords. The keywords will contain "animals" and the term for any animal types which may be used in the work unit (i.e., Guinea Pigs, Rats).

A sample of publicly accessible information on animal use in the DoD is included in Appendix F.

2. Data collected from DoD animal research projects, including those funded by non-RDT&E programs, will be loaded into a single database. Projects will be entered into this database by the end of second quarter FY95. Simultaneously, the system for developing public accessibility through creation of a Home Page on Internet and/or connections to the FEDRIP database will have been completed.

3. Loading, testing, and final evaluation of the connections for public access will be completed by end of third quarter FY95 (June 1, 1995).

4. The Department's goal is to have at least 95% of FY94 animal-based research summaries in the database ready for public access by October 1, 1995.

In addition to these efforts for FY94, the DoD plans to create an ongoing capability to capture the data in DTIC's work unit summaries and transport them into the publicly accessible database. The data collection is planned to consist of automated extraction of data from DTIC work unit summaries and automated entry of these data into the publicly accessible database. In future years, work unit summaries are expected to be submitted for both RDT&E and non-RDT&E activities involving animal use. The Department plans to complete arrangements for continued maintenance and updating of the database by October 1, 1995.

## OVERSIGHT OF DoD ANIMAL CARE AND USE PROGRAMS

## Section



This section responds to the House Armed Services Committee (HASC) report that "directed the Secretary to require a search of federal research in progress databases before new research is approved." Additionally, the HASC expected that "animal advocates be appointed as the bona fide community member of the IACUC at each DoD facility." The committee further expected the Secretary of Defense to provide "a plan to improve community representation on DoD research facility IACUCs" (H.R. 4301, 1994). Information on the mechanisms and procedures for oversight, management and direction of research planning and the actual conduct of research requiring the use of animals as subjects will be presented. For the purposes of this report and consistent with the President's National Defense Budget Request, research is defined as those congressionally authorized science and technology (S&T) based activities - *Title II, Research, Development, Test and Evaluation* (RDT&E) - of the Military Departments, and for which funds are appropriated, within program elements 6.1 (Basic Research), 6.2 (Exploratory Development) and 6.3 (Advanced Development).

The mechanisms detailed here show a clear and long-standing commitment by the Department of Defense (DoD) to manage its biomedical research and clinical programs in a systematic, comprehensive, and effective manner. Individual programs are driven by specific mission requirements, and are subjected to a thorough, stratified review and analysis prior to commitment of funds. The DoD will use animals when necessary to complete its mission, and in a way that is in compliance with applicable laws, regulations, and guidelines.

### III.1 DETERMINATION OF DoD NEEDS FOR ANIMAL RESEARCH

Determining research needs and research plans is a comprehensive process integrated into DoD's planning, programming and budgeting processes. Integral elements of these processes are the Department's Research and Development Descriptive Summaries submitted to Congress in justification of the budget request. These summaries provide the Office of the Secretary of Defense, the Office of Management and Budget, and the Congress with significant detail of every research project's past accomplishments, planned accomplishments and future plans.

Each Defense research laboratory tailors its organization, staffing, and related infrastructure within available resources to best meet its science and technology mission and to support each Commander's accountability, responsibility and authority. Although the specific procedural elements and processes of individual protocol review may differ somewhat from facility to facility, DoD researchers will use a comprehensive DoD Standard Protocol Format as a basis to justify and



document all proposed animal use (Appendix C). This Standard Protocol Format was developed in response to the DoD Inspector General's recommendations for the use of animals in DoD medical research facilities. The Standard Protocol Format is designed to solicit specific information essential to ensuring a complete and thorough Institutional Animal Care and Use Committee (IACUC) review for all animal use proposals. The general submission, review and approval process is summarized as follows:

An investigator develops a research protocol in support of Departmental S&T guidance and other supplementing guidance developed within the chain-of-command, both external and internal to the laboratory. Augmenting the formal S&T coordination and review process is a literature search to verify non-duplication of previous or on-going research. Previously, this search was performed only on the Defense Technical Information Center (DTIC) database. DTIC maintains a database of ongoing and completed research at the work unit level of detail. In response to the HASC request, the Standard Protocol Format requires that "a search of Federal Research in Progress (FEDRIP) and DTIC databases or their equivalent is required for DoD-funded research. An additional search of the scientific literature (MEDLINE, GRATEFUL MED, MEDLARS, AWIC, etc.) is highly recommended." Review and certification that this requirement has been met are integral elements of the review and approval process for initiating a research project. If animal use is planned for the intended research, the principal investigator must prepare an animal protocol request for the local IACUC. In addition to the DTIC and FEDRIP search, the Standard Protocol Format requires detailed information regarding results and dates of other on-line database searches (e.g., AWIC, AGRICOLA, CAAT, MEDLINE) that deal with alternatives to painful procedures. Additional pertinent knowledge and information on the proposed study are gained through review of the scientific literature and participation in scientific meetings, symposia, and workshops detailing other on-going or completed research.

Since protocols require the utilization of Defense resources, individual protocols are reviewed for factors such as military relevancy, necessity, scientific merit, and relative research priority. Such reviews are normally conducted within the laboratory's command-and-control structure and are routinely characterized by the features of peer review systems.

DoD IACUCs carefully review research proposals involving the care and use of animals for numerous factors, including but not limited to (a) the study is based on sound scientific principles, (b) the number of animals used is the minimum required to achieve the purpose, (c) the phylogenetically lowest species of animal is selected as the appropriate model, (d) there is appropriate use of analgesics and anesthetics, if required, and if not used, there is adequate scientific justification, (e) the research is not unnecessarily duplicative, (f) the personnel conducting the research are qualified by training and experience to conduct the research, and (g) the scientific question to be answered is of sufficient importance to warrant the use of animals. Additionally, detailed information regarding methodology, techniques,

schedules, etc., is required, greatly facilitating a comprehensive and thorough review by IACUCs.

### **III.2 OVERSIGHT OF ANIMAL CARE AND USE PROGRAMS AND FACILITIES**

There are three principal vehicles for oversight of animal care and use programs at DoD research facilities: Major DoD Activities and Service Commands, the IACUC, and the American Association for Accreditation of Laboratory Animal Care (AAALAC).

#### **III.2.1 Military Departments**

Each Military Department has a component or components responsible for oversight and review of its research facilities and animal care and use programs. Periodic reviews, site visits, and inspections are conducted formally and reports are prepared.

The Army's ultimate oversight responsibility is divided between two major commands; the U.S. Army Medical Command, and the U.S. Army Materiel Command. In the U.S. Army Medical Command, programmatic guidance and site visits are performed by specialty trained laboratory animal medicine (LAM) veterinarians in the Headquarters, U.S. Army Medical Research and Materiel Command (USAMRMC), and the U.S. Army Medical Department Center and School (Veterinary Programs Manager). In the U.S. Army Materiel Command, oversight is by a specialty trained LAM veterinarian assigned to the U.S. Army Chemical and Biological Defense Command. Ultimate responsibility for laboratory animal care and use programs in the Navy resides in the Office of the Surgeon General of the Navy. Oversight is accomplished by a specialty trained LAM veterinarian assigned to the Naval Medical Research and Development Command, by the Health Services Education and Training Command (Clinical Investigations), and the Inspector General at the Naval Bureau of Medicine and Surgery. Air Force oversight responsibility rests with the HQ, Air Force Medical Operations Agency, Clinical Investigations & Life Sciences Division, Office of the Air Force Surgeon General, and with the Office of the Director of Medical Inspection, Air Force Inspection Agency.

#### **III.2.2 IACUC**

A common review element for all DoD animal-based research is the IACUC review of the research proposal or protocol. DoD Directive 3216.1 requires all DoD facilities using animals in research to comply with the Animal Welfare Act (AWA). The AWA requires the Chief Executive Officer to appoint an IACUC, qualified through the experience and expertise of its members, to assess the research facility's animal program, facilities, and procedures. Additionally, the AWA requires that IACUCs have a minimum of three members: an appropriately qualified chairman; at least one member not affiliated with the institution in any way other than as a member of the Committee; and a veterinarian with training or experience in

laboratory animal medicine and science. Each DoD IACUC is currently chaired by an individual with credentials and experience appropriate to the post, typically a senior physician, scientist, or veterinarian. The revised DoD Directive 3216.1 (1995) (Appendix A) clarifies the composition, membership, and training requirements of the IACUC. The changes address the HASC request to improve community representation and to appoint animal advocates to DoD IACUCs, and are consistent with a recommendation of the Inspector General's report of February 1994 (Appendix E). The revised Directive (1995) increases the minimum size of all DoD IACUCs from three to five, which is in concert with the National Institutes of Health (NIH) Office for Protection from Research Risks (OPRR) model. In addition, it specifies that:

"...there shall be at least one non-scientific member on the IACUC. In addition, there shall be at least one member representing the general community interest who is nonaffiliated with the research facility. The nonaffiliated member and the non-scientific membership can be filled by the same person. To ensure community representation at each meeting and inspection, an alternate to the nonaffiliated member shall be designated for all IACUCs having a single nonaffiliated membership."

This Directive exceeds the requirements of the AWA and is further strengthened by The DoD 1995 Policy Letter (Appendix B) that directs a minimum of eight hours of training for the nonaffiliated members. All IACUCs must strive towards implementation of these requirements by October 1, 1995. Significantly, eleven IACUCs currently have more than one nonaffiliated member or alternate to maximize community involvement. Each IACUC has at least one Doctor of Veterinary Medicine with training or experience in laboratory animal science and medicine and serves as an animal advocate. The U.S. Army Veterinary Corp's formal postgraduate training program in Laboratory Animal Medicine provides didactic training in IACUC composition, function, and regulatory requirements. This training also prepares them to serve as animal advocates.

It is a proactive Department policy that nonaffiliated members are encouraged to perform unannounced site visits of animal care facilities in addition to full participation in all discussions and votes on all research proposals. Twenty-five unannounced visits to Department animal facilities by nonaffiliated members of DoD IACUCs were reported in FY94.

The IACUC has statutory responsibility for reviewing the facility's animal care and use program and inspecting the animal facilities on a semiannual basis. Consequently, at least once every six months, each IACUC performs an in-depth review of the animal care and use program and inspects the animal facilities. To facilitate these inspections the DoD has developed and implemented (DoD 1995 Policy Letter) a standardized semiannual program review checklist that details the requirements of the review. This policy is consistent with the recommendations of the DoD Inspector General's report of February 1994. The IACUC prepares written reports of its evaluations and submits them to the Institutional Official, usually the

facility commander. Reports specifically address compliance with the AWA, and identify any departures from the Act to include an explanation for the departure. The report must distinguish between significant and minor deficiencies and provide a schedule for resolution of significant deficiencies.

All DoD IACUCs document their meetings and activities, including the results of inspections, complaints, actions, and training. They are empowered to review and investigate concerns involving the care and use of animals at the research facility resulting from complaints received from the public or in-house workers, or from reports of noncompliance received from laboratory personnel. To facilitate the reporting and resolution of complaints or concerns, facilities commonly place signs in public areas and in animal study areas advising both the public and personnel who work with animals how to contact members of the IACUC, facility commanders, and/or the Inspector General (IG) whenever questions concerning humane care and treatment of animals arise. DoD facilities have developed a wide variety of proactive and innovative mechanisms to both inform the public on how to contact responsible individuals as well as programs to ensure that those who work with animals are fully apprised of the requirement to provide humane and ethical care (Appendix G). Additionally, IACUCs make recommendations to the Institutional Official regarding any aspect of the research facility's animal program, facility, or personnel training; review and approve, require modification to, or withhold approval of new research protocols involving the use of animals; review and approve, require modification to, or withhold approval of proposed significant changes regarding the care and use of animals in ongoing research protocols; and suspend an activity involving animals when they determine that the activity is not being conducted in accordance with the approved protocol.

### III.2.3 Training

The DoD provides extensive veterinary and animal care services for DoD facilities. Veterinarians with specialty training in LAM direct programs for animal care and use throughout the Department. They serve as a valuable resource to the research staff and the IACUC to ensure that all research methods and maintenance procedures are consistent with the latest principles of animal medicine, and current interpretations and implementing regulations of the AWA. The DoD sponsors formal post-doctoral training programs for veterinarians in LAM, including a nationally recognized in-house four-year LAM residency program culminating in specialty board eligibility for certification in the American College of Laboratory Animal Medicine (ACLAM). DoD Veterinarians also attend various university post-graduate LAM training programs resulting in a masters degree or Ph.D. Approximately 25% of the current members of the ACLAM, the veterinary specialty most closely associated with animal welfare and laboratory animal care and use, received either all or part of their training in DoD-sponsored LAM training programs. In addition, the DoD trains animal care specialists (Military Occupation Specialty 91T) to assist in the daily management, care and treatment of laboratory animals. Over the last 27 years, the DoD has trained over 3000 animal care specialists. Additionally, DoD research institutions sent appropriate staff to a variety

of seminars and workshops sponsored by the National Institutes of Health, other federal agencies, and private institutions dedicated to the proper care and use of research animals.

The DoD provides detailed informational and instructional material to all members of the IACUC, including nonaffiliated members to ensure that each is fully cognizant of the numerous responsibilities of IACUC members under the provisions of the AWA. The DoD is committed to providing at least eight hours of training and instruction for IACUC members in animal care and use, regulatory, and other pertinent issues. Formal training on animal care and use issues is provided to all appropriate personnel in Department research laboratories in accordance with the provisions of the AWA. Examples of training or materials currently provided to IACUC members are detailed in Appendix H.

### **III.2.4 AAALAC**

AAALAC is a nonprofit organization chartered to promote high quality standards of animal care, use, and welfare through the accreditation process. The AAALAC accreditation process provides scientists and administrators with an independent, rigorous assessment of the organization's animal care and use program. To increase accountability and tracking, a centralized DoD point of contact and database for AAALAC information is being established to enhance monitoring, reporting, and facilitation of the AAALAC accreditation process. An in-depth discussion of the AAALAC accreditation process and a profile of the DoD's participation is provided in Section IV.

### **III.2.5 Community Visits**

Individuals or groups wishing to visit Department facilities need to comply with certain procedural guidelines. All DoD facilities are served by a public affairs office, either at the facility, post, or base. Visits by the public or the press should be arranged and coordinated through the appropriate public affairs office. DoD facilities are visited by various special interest groups including community and civic groups; animal welfare or animal advocates groups or individuals; dignitaries, academics and teachers; local, state, and national politicians; congressional members and staff; elementary to post-doctoral students; print and electronic journalists and authors; etc. Consequently, a diverse range of individuals visit and observe the quality of Department facilities.

### **III.2.6 Office for Protection from Research Risk Oversight**

Institutional compliance with *The Public Health Service Policy on Humane Care and Use of Laboratory Animals* (PHS Policy) is a prerequisite for granting or continuation of NIH intramural and extramural funding. The formal vehicle for compliance with the PHS policy is an "Animal Welfare Assurance" negotiated between individual institutions and the OPRR. The principal references for the negotiation of an OPRR "assurance" are the Health Research Extension Act of 1985

(Public Law 99-158), the Animal Welfare Act, and the NIH *Guide for the Care and Use of Laboratory Animals*.

### III.2.7 Additional Oversight

Within the DoD, individuals may raise animal welfare concerns with the IACUC, facility commanders, the IG, attending veterinarian, or in other ways both within and outside (e.g., Waste, Fraud and Abuse Hotline) the formal chain of command. Procedures to enhance and facilitate these mechanisms have been implemented in DoD facilities.

The function of the IACUC and the role of an ombudsman is augmented by the Department's IG. An ombudsman is defined by Webster's dictionary as a government official charged with investigating citizens' complaints against the government. The Humane Society of the United States, a witness at the April 7, 1992 hearing on The Use of Animals in Research by the Department of Defense before the House Armed Services Committee, offered the ombudsman program at the Massachusetts Institute of Technology as an example of a model program. This program consists of an ombudsman assigned to the university president's office to hear complaints regardless of the nature. These include, but are not limited to, personnel complaints, sexual harassment, animal welfare, etc. The DoD assigns this responsibility to its IG and respective Inspectors General of the Military Departments. In addition, military bases and large organizations on military bases have their own Inspectors General who fulfill this function.

Oversight of extramural (contract) animal-based research is provided for in the revised DoD Directive 3216.1 (1995). It states that

a. "all extramural research proposals using live animals shall be administratively reviewed by a DoD veterinarian trained or experienced in laboratory animal science and medicine before grant or contract award."

b. "the most recent USDA inspection reports are provided or obtained for the facility under consideration for a research contract or grant using animals, and that during the term of the award, the most recent USDA inspection reports be reviewed on an annual basis."

c. "a DoD veterinarian trained or experienced in laboratory animal science and medicine shall conduct an initial site visit to evaluate animal care and use programs at contract facilities conducting DoD-sponsored research using nonhuman primates, marine mammals, dogs, cats, or proposals deemed to warrant review. The initial site visit shall occur within 6 months of when the facility has taken delivery of the animals under DoD contract or grant award. Any facility receiving a DoD-funded grant or contract for animal-based research shall notify the DoD component sponsor and shall have a site inspection within 30 days of notification of loss of AAALAC accreditation for cause, or notification that the facility is under USDA investigation. Site inspections for cause shall evaluate and ensure the adequacy of

animal care and use in DoD-sponsored programs, and provide recommendations to the sponsoring DoD component about continued funding support of the research."

As directed by DoD Directive 3216.1, all nonhuman primate protocols receive an additional centralized review external to the research facility.

### **III.3 CHAIN OF COMMAND OVER ANIMAL CARE AND USE PROGRAMS**

The chain of command is designed to resolve problems at the lowest possible level. It provides control and communication between various components of organizations. Each link in the chain of command is a level of responsibility and authority that extends from the President of the United States, as Commander in Chief, down to each supervisory level. Different levels within the chain have different responsibilities and authority. Each level in the chain is responsible for a lower level and accountable to a higher one. Every individual in the military is part of the chain of command.

### **III.4 AVOIDANCE OF UNINTENDED DUPLICATION OF RESEARCH**

Both the DoD and the Congress have a long history of concern about the potential for unintended duplication of Defense research. Within the past decade, the Department has initiated significant improvements in its mechanisms for coordination, joint planning and review of its research programs.

Congress, in 1981, expressed concerns about the potential for unnecessary duplication of biomedical research among the Military Departments (H.R. 96-1317). This resulted in the DoD proposing an Armed Services Biomedical Research Evaluation and Management (ASBREM) Committee to coordinate biomedical research planning and the conduct of biomedical research among the Military Departments. Congress fully endorsed and built upon this proposal by establishing DoD Lead Agencies for major elements of the biomedical research programs for which there were either no, or very few, service-unique requirements (H.R. 97-332). For example, the Army was designated as the DoD Lead Agency for military infectious disease and combat maxillofacial research while the Navy was designated DoD Lead Agency for preventive and emergency dentistry research. The ASBREM Committee established Joint Technology Coordinating Groups (JTCGs), consisting of directors of biomedical research programs and representatives of biomedical research laboratories, to coordinate all DoD biomedical research planning and execution. The ASBREM Committee process has proven to be highly effective at eliminating unnecessary duplication of biomedical research.

The ASBREM Committee process became the model for joint DoD coordination initiatives. Responsibility for joint coordination, planning, execution and review of the Departments' S&T programs was assigned to joint oversight bodies: the Joint Directors of Laboratories (JDL), the ASBREM Committee, the Training and Personnel Systems Science and Technology Evaluation and Management (TAPSTEM) Committee, and the Joint Engineers. The resulting

technology area responsibilities are shown in Figure III-1. Joint S&T oversight bodies are assisted in execution of their responsibilities by subordinate S&T coordinating groups that are focused on coordination of specific technology areas. For example, the ASBREM Committee is supported by the JTCGs (Figure III-2) and the JDL is supported by separate technology panels.

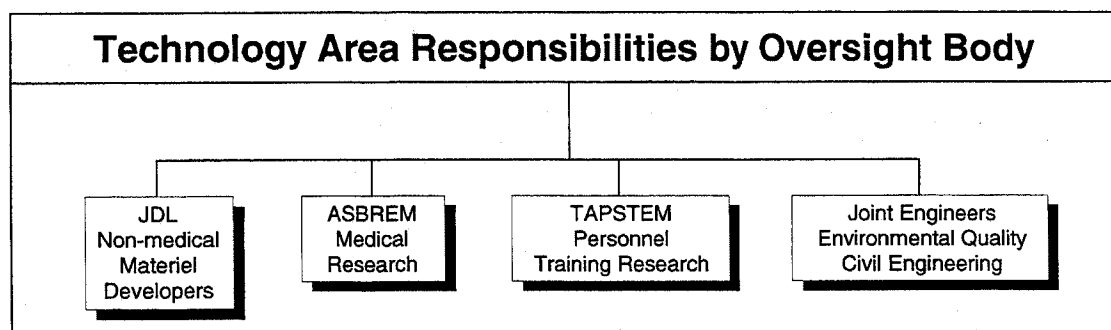


Figure III-1. DoD Technology Area Responsibilities

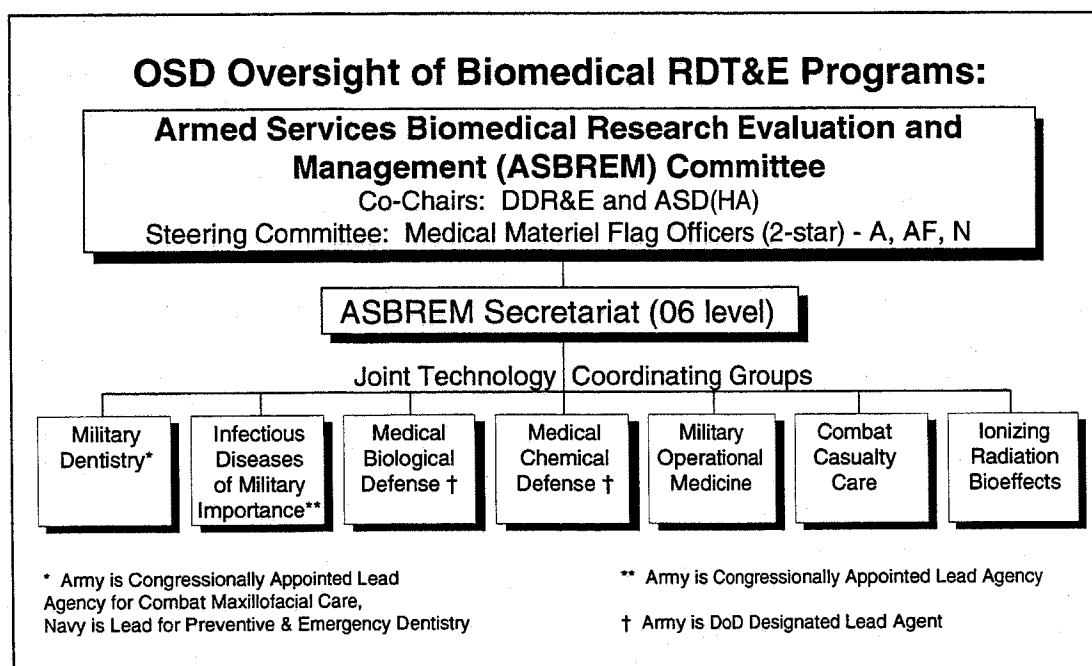


Figure III-2. Structure of Armed Services Biomedical Research Evaluation and Management Committee

In addition to these formal coordination and review processes to eliminate unintended duplication of research, there are a number of less formal mechanisms that provide significant disincentives for research duplication. Competition, both



in-house and extramural, for research support is a prominent feature of S&T; each year large numbers of scientifically meritorious research proposals cannot be funded due to shrinking resources and funding shortages. The professional stature of individual scientists or engineers among their peers is measured in proportion to their individual and original contributions to the scientific literature. There is little if any reward for unnecessarily duplicating the work of others; such actions often have significant negative impacts on how the scientist or engineer is viewed by peers and on the ability to secure research support. Additionally, within the DoD civilian personnel system, scientists' and engineers' pay grades are determined in part by the level of individual scientific and technological contributions. One outcome of research is publication of a manuscript in a professional journal or presentation to a professional meeting (Appendix I). Peer-reviewed journals critique the research during the review process leading to an overall enhancement of the research process as well as validating the scientific merit and necessity of the research. These less formal, relatively unquantifiable, disincentives substantially augment and buttress the Department's formal mechanisms for regulating and avoiding unnecessary research duplication within its S&T programs.

### **III.5 AVOIDANCE OF UNNECESSARY RESEARCH**

The same factors that effectively prevent unwarranted duplication of research are also applied to prevent unnecessary research. Additionally, through Cooperative Research and Development Agreements, the Department has increased its emphasis on leveraging and exploiting for Defense needs, S&T investments by other federal agencies, U.S. industry, and academic institutions, as well as by the international scientific community. Past descriptions of Defense S&T "spin off" have been supplanted by programs intended to "spin-on" accomplishments by others as well as to optimize the dual-use potential of the Defense S&T investment. The foundation of Defense S&T strategy is the application of S&T accomplishments to sustain Defense technological superiority through efficient and responsive modernization of our warfighting capabilities.

### **III.6 SUMMARY**

Biomedical research using animals is highly structured and regulated in the United States, being governed by numerous laws, regulations, and policies. Consequently, the DoD has a number of stratified formal and informal mechanisms for reviewing, regulating, and executing its biomedical research mission and animal care and use programs. Research performed by the DoD is carefully reviewed by various offices, committees, and program managers before it is funded or implemented. These reviews serve to determine the necessity to the mission, provide oversight of animal care and use, and avoid unnecessary or unintended duplication of research. Over the past decade the DoD, in concert with the Congress, has streamlined and greatly improved coordination of its S&T activities to avoid unnecessary duplication and provide a focused program of research responsive to the DoD's unique needs. Individual IACUCs provide oversight of animal care and use programs and research. Additionally, IACUCs provide training and information

about animal care and use, and assure the humane use of animals in research. Each DoD facility's IG is an effective means for investigation of concerns about the necessity of animal use, as well as the ethical treatment and humane care of animals used in DoD research.

# AAALAC ACCREDITATION OF DoD LABORATORIES

## Section IV

This section responds to the National Defense Authorization Act for Fiscal Year 1995 Conference Report S. 2182, which states that "... the conferees urge the DoD to seek AAALAC accreditation of all DoD animal facilities as expeditiously as possible." The Department of Defense (DoD) recognizes the benefits of the American Association for Accreditation of Laboratory Animal Care (AAALAC) accreditation. With the publication of the Joint Regulation on the Use of Animals in DoD programs, June 1, 1984 (AR 70-18), the DoD implemented more stringent animal care and use requirements than those required by statute. The Joint Regulation established uniform procedures, policies and responsibilities for the use of animals in the DoD. This year the DoD has elevated the requirement with the current DoD Directive 3216.1 (1995) which states that "all DoD laboratories that maintain animals for use in research, testing or training shall apply for AAALAC accreditation." The Joint Service Regulation also cites the National Institutes of Health (NIH) publication, *Guide for the Care and Use of Laboratory Animals*, which is the principal document used by AAALAC in its accreditation process. The animal care and husbandry standards and requirements contained in the Guide are designed to provide an environment that ensures proper care and humane treatment are given to all animals used in research, testing, and education. This care requires scientific and professional judgment based on knowledge of the husbandry needs of each species, as well as the special requirements of the research program.

### IV.1 AAALAC ACCREDITATION

AAALAC accreditation is widely accepted by the scientific community, and viewed as an extremely desirable feature of the Department's animal care and use programs. The Association is highly respected as an independent organization that evaluates the quality of laboratory animal care and use. Accreditation covers all aspects of animal care to include institutional policies; laboratory animal husbandry; veterinary care; facility physical plant; support facilities; and special areas of breeding colony operations and animal research involving hazardous agents such as radioactive substances, infectious agents, or toxic chemicals.

The non-biased, independent, external peer review which is fundamental to continuing AAALAC accreditation is valuable to any size program. AAALAC findings highlight program strengths and identify potential weaknesses. Laboratories maintaining accreditation demonstrate a high degree of accountability and program excellence. AAALAC standards stress the appropriate appointment, composition, and empowerment of an Institutional Animal Care and Use Committee (IACUC). This Committee is responsible for monitoring and evaluating all aspects

of the institution's program which uses animals for teaching and/or research purposes. The scope of IACUC functions is addressed in Section III of this report.

## IV.2 DoD PROGRAM REVIEWS

DoD utilizes external peer review for the evaluation of many of its programs, such as drug screening laboratories, and review of military medical facilities by the Joint Commission for Accreditation of Health Organizations. At the same time, DoD recognizes the diversity of mission operations and global reach of the military mission. There are situations where external peer reviews are not cost effective due to the remote locale, limited scope of operations, or host nation sovereignty. In these cases, equivalency standards can apply and be effectively monitored. The Joint Service Regulation and Service-conducted inspections of facilities implement the requirements of the Animal Welfare Act and the NIH *Guide for the Care and Use of Laboratory Animals*. As noted in the Office of the Inspector General, Department of Defense (OIG-DoD) *Review of the Use of Animals in Department of Defense Medical Research Facilities*, February 1994, best practices were found in both AAALAC and non-AAALAC accredited DoD programs.

The DoD is committed to accrediting its research programs. In fact, of the two programs identified by the DoD IG in their 1994 report as "not substantially in compliance" with DoD regulations and the Animal Welfare Act, both are now accredited by AAALAC. In recognition, the conference report on the FY95 National Defense Authorization Act stated that "the conferees applaud the Department's expeditious efforts to gain accreditation for these facilities."

## IV.3 AAALAC ACCREDITATION STATUS FOR U.S. DoD PROGRAMS

There are 34 separate facilities in the U.S. that maintain animals for research, testing, or training for the DoD. Table IV-1 shows that of the 34 DoD programs, 79% are accredited by AAALAC. This compares very favorably with the accreditation rate for the 1,433 United States Department of Agriculture (USDA) registered animal facilities; 583 or 41%, are accredited by AAALAC. Of the seven DoD programs in the U.S. that are not accredited, four have applied and three are preparing applications. In addition, there are four DoD animal use programs that share DoD AAALAC accredited facilities. These programs are small detachments that are assigned to DoD bases and therefore share their animal care and use facilities. Appendix J provides additional information on AAALAC accreditation by program.

Table IV-1. DoDAAALAC Accreditation Status

AAALAC Status	U.S. DoD Programs	Overseas DoD Programs
Accredited	27	1
Application submitted	4	3
Application Pending	3	0
Total	34	4

The AAALAC philosophy of accreditation is steadily evolving from an emphasis on physical facilities (engineering standards) to a more comprehensive evaluation of the total laboratory animal care and use program (performance standards). Facilities are still an important consideration in the accreditation process, but are no longer the paramount element. Consequently, research units that were previously regarded as unaccreditable until major facility renovations or upgrades were completed are now actively pursuing AAALAC accreditation on the basis of comprehensive, high quality laboratory animal care and use programs. The lack of accreditation does not imply that animals are exposed to unhealthy conditions. The OIG-DoD's *Review of the Use of Animals in DoD Medical Research Facilities*, February 1994, confirmed the effectiveness of animal husbandry programs in DoD facilities and concluded that although not all programs were AAALAC accredited, animals in DoD programs were maintained in healthy environments and treated humanely.

#### **IV.4 AAALAC ACCREDITATION STATUS FOR DoD OVERSEAS PROGRAMS**

There are four programs using animals outside the United States. Previously the DoD had not sought AAALAC accreditation outside of the U.S. In foreign countries, the accreditation process is complicated by issues of sovereignty. Local governments have their own regulations and policies that must be considered. Renegotiation of various agreements may be involved in construction or renovation projects. Despite these and various other impediments, the DoD is committed to accrediting its overseas laboratories as quickly as possible. Currently, the three overseas programs have submitted applications for accreditation and one program is accredited. The Naval Medical Research Detachment Lima, Peru, is the first laboratory in South America to receive AAALAC accreditation.

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## **DoD ANIMAL USE AND COST PROFILES BY RESEARCH CATEGORY**

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## **Section V**

This section responds to the House Armed Services Committee's request that "the Secretary of Defense submit a comprehensive annual report on animal cost and use programs including in-depth profiles for animal research with information about cost, kinds and numbers of animals used, and a pain evaluation" (H.R. 4301, 1995).

### **V.1 METHODS**

Information was solicited and received from Department of Defense (DoD) agencies and military Commands, organizations, and activities involved in the performance and/or funding of animal care and use programs located both inside and outside of the United States. This included extramural contractors and grantees that performed animal-based research. For the purposes of this reporting requirement, an intramural program represents research performed at a DoD facility and funded by either DoD or non-DoD funds. An extramural program represents research performed by a contractor or grantee which is funded by the DoD.

#### **V.1.1 Animal Use Profiles**

The animal use profiles prepared for this report are consistent with the reporting information and data provided to the U.S. Department of Agriculture (USDA) using the Animal Plant Health Inspection Service (APHIS) Form 7023. In addition, this report contains comprehensive information on all other animals (i.e., mice, rats, birds) used that are not required in reports to the USDA.

For the purposes of this reporting requirement, an animal was defined as any whole nonhuman vertebrate, living or dead, excluding embryos, that was used for research, development, test, and evaluation (RDT&E), clinical investigations, diagnostic procedures, and/or instructional programs. Only live animals or whole dead animals, as defined, that were either on hand in the facility or acquired during fiscal year (FY) 1994 were included. Animal organs, tissues, cells, blood, fluid components, and/or by-products purchased or acquired as such animal/biological components are not reported. This definition does not include animals used or intended for use as food for consumption by humans or animals, animals used for ceremonial purposes, nor military working animals and their training programs.

A single animal was counted only once in determining the number of animals used during the fiscal year for a particular work unit or protocol. This does not refer to the number of times an individual animal is injected, manipulated,

handled, or administered medication and/or experimental compounds within a given work unit, protocol, or program. Animals on hand during FY94, but not actually used during the fiscal year, are not included in this number.

### V.1.2 Animal Use Categories

All DoD agencies and military Commands, organizations, and activities involved in the performance and/or funding of animal care and use programs reported animal work by the category that best describes the general purpose of the animal use. If these categories did not describe the animal use within a particular work effort, the animal was placed under the Other category. The eight general categories and 23 specific subcategories are listed in Table V-1. In-depth information on specific activities performed within a subcategory is presented in Appendix K. The medical research categories correspond to the Armed Services Biomedical Research Evaluation and Management (ASBREM) Committee Joint Technology Coordinating Group Medical Research Areas. Non-medical categories consist of RDT&E programs performed outside the ASBREM Committee medical oversight. Clinical Investigations studies were performed under the auspices of the Assistant Secretary of Defense for Health Affairs and the military services medical departments through Major Force Program 8 funding. These studies were usually in support of graduate medical education training programs located at the major military medical centers.

Table V-1. Animal Use Categories

<b>MEDICAL (M)</b>
M1: Military Dentistry
M2: Infectious Diseases
M3: Medical Chemical Defense
M4: Medical Biological Defense
M5: Human Systems Technology
M6: Combat Casualty Care
M7: Ionizing Radiation
M8: Other Medical RDT&E
<b>NON-MEDICAL (N)</b>
N1: Physical Protection
N2: Physical Detection
N3: Offensive Weapons Testing
N4: Other Non-Medical RDT&E
<b>CLINICAL INVESTIGATIONS (C)</b>
C1: Clinical Medicine
C2: Clinical Surgery
C3: Other Clinical Investigations
<b>TRAINING AND INSTRUCTIONAL (T)</b>
T1: Training, Education, and/or Instruction for Personnel
T2: Other Training/Instruction
<b>ADJUNCTS AND ALTERNATIVES TO ANIMAL STUDIES (A)</b>
A1: Adjuncts to Animal Use Research
A2: Alternatives to Animal Investigation
A3: Other Alternatives/Adjuncts
<b>CLASSIFIED SECRET OR ABOVE STUDIES (S):</b>
Animals on studies classified Secret or above
<b>ANIMAL BREEDING STOCK (B):</b> Animals maintained for breeding
<b>OTHER ANIMAL USE CATEGORIES (O):</b> Other animal use purposes

### V.1.3 USDA Pain Categories

The USDA requires that all institutions using any regulated animal for research, testing, training, or experimentation register with the USDA as a research facility and submit an annual report. This annual report presents the number of regulated animals used and the type of pain, if any, the animals were exposed to.

The USDA has developed three pain categories for its reporting requirement (Table V-2). All animals herein reported are assigned to one of the three USDA pain categories; this includes animals that are not regulated by the USDA. The USDA requires that any reporting facility that uses procedures producing unalleviated pain or distress file an explanation of the procedures with its annual APHIS report.

The animals reported in Column C of the USDA report are those used in procedures that are not painful. Procedures performed on these animals are those that are usually conducted on humans without anesthesia or analgesia. Examples include most blood sampling techniques (excluding intracardiac and periorbital blood sampling), injections and tattooing.

The animals reported in Column D of the USDA report are those that experience pain in which appropriate anesthetics, analgesic or tranquilizing drugs were used. Examples include anesthesia for surgical procedures or catheter placement, and analgesia during recovery from surgery.

The animals reported in Column E of the USDA report are those that experience more than slight or momentary pain or distress that cannot be alleviated by drugs. Examples of procedures where drugs were not used because they would have adversely affected the procedures, results or interpretation of the research, surgery or test include some infectious disease studies and toxicology studies.

All procedures that involve animals in Columns D or E are extensively reviewed during the protocol approval process. A veterinarian with experience and/or training in laboratory animal medicine must review all procedures that could cause pain and distress in animals. In addition, the primary investigator must write a justification for all procedures for animals in Columns D and E. The DoD standard protocol states, "Procedures causing more than transient or slight pain that are unalleviated, must be justified on a scientific basis in writing by the primary investigator. The pain must continue for only the necessary period of time dictated by the experiment, and then be alleviated, or the animal humanely euthanized." Moreover, the primary investigator must sign an assurance statement that alternative procedures are not available, and the IACUC must review and approve all procedures before the study begins.

**Table V-2. USDA Pain Categories**  
(USDA APHIS form 7023)

**USDA COLUMN C**

Number of animals upon which teaching, research, experiments, or tests were conducted involving no pain, distress, or use of pain-relieving drugs.

**USDA COLUMN D**

Number of animals upon which experiments, teaching, research, surgery, or tests were conducted involving accompanying pain or distress to the animals and for which appropriate anesthetic, analgesic, or tranquilizing drugs were used.

**USDA COLUMN E**

Number of animals upon which teaching, experiments, research, surgery, or tests were conducted involving accompanying pain or distress to the animals and for which the use of appropriate anesthetic, analgesic, or tranquilizing drugs would have adversely affected the procedures, results, or interpretation of the teaching, research, experiments, surgery, or tests.



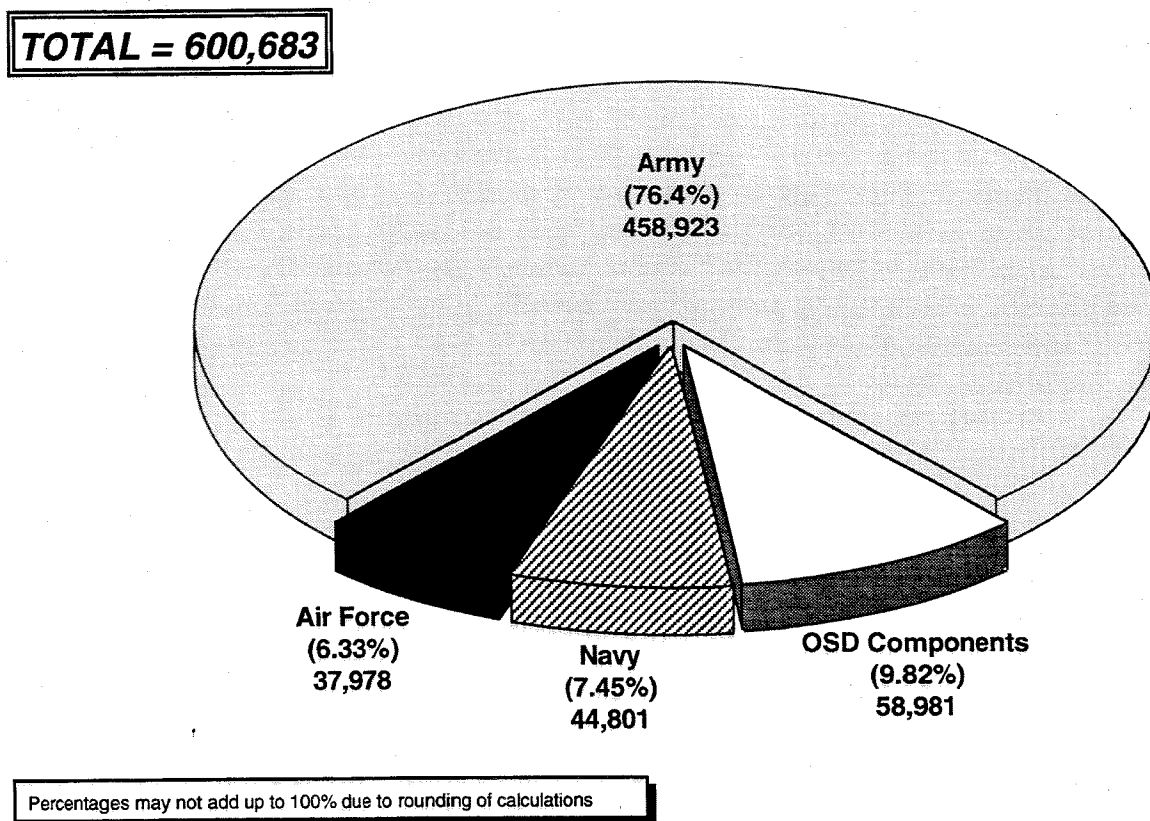
## V.1.4 Animal Cost Profiles

This report provides the total cost by research category for DoD-funded animal use programs. The total cost by category reported represents the collation of estimates provided by each of the agencies, military commands, organizations, and activities. The funding amounts provided report the total dollars applied to all animal-based research in work units, protocols, or contracts and not simply the portion of funding within an effort used for the direct purchase and care of animals. If any portion of a work unit, protocol, or contract is involved in animal-based research, the entire cost of the work unit is included. Therefore, reporting cost in this manner may overstate the total cost of animal work. Overhead costs for the animal work may not be included if the sum of the work unit, protocol, or specific program efforts do not provide the costs of general animal care and support. Military salaries are not included in these cost computations.

## V.2 RESULTS/DISCUSSION

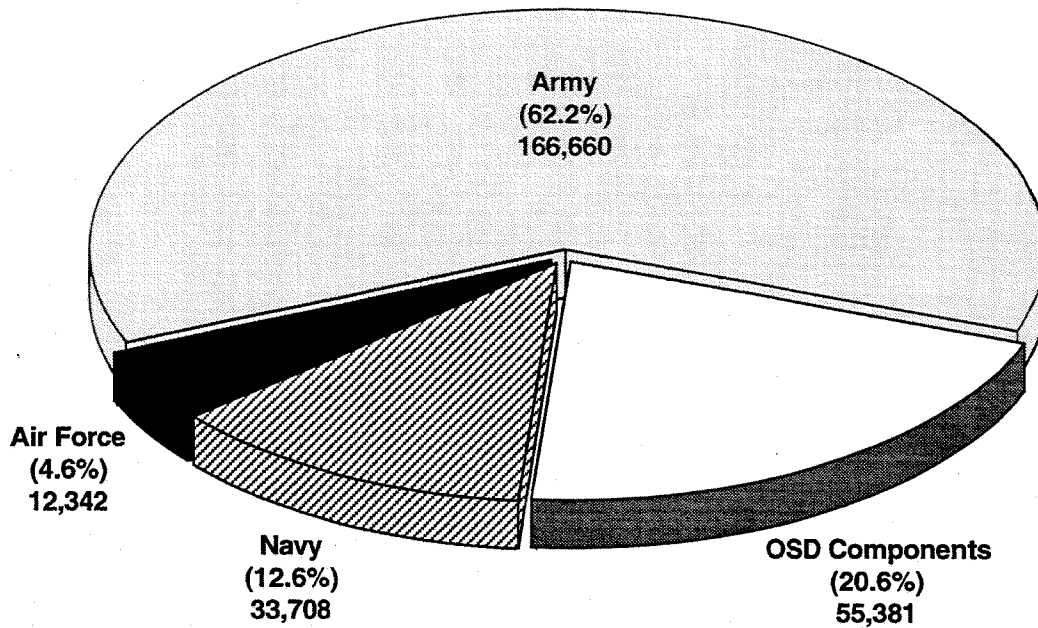
### V.2.1 Animal Use by Service

Information concerning total DoD utilization of animals by each service is presented in Figure V-1. Figures V-2 and V-3 show the intramural and extramural animal use by service, respectively.



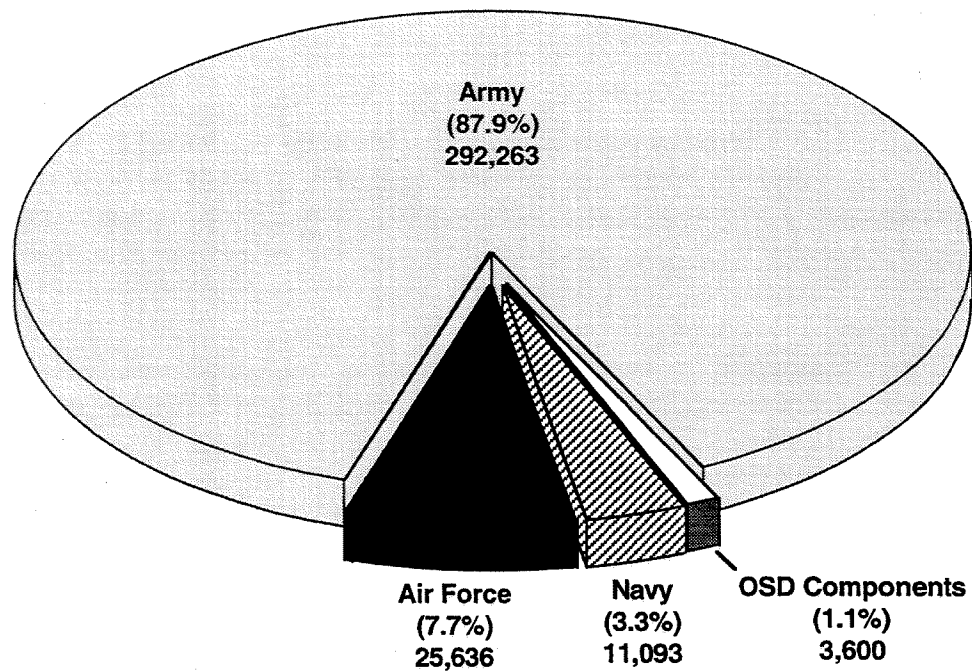
**Figure V-1.** Total DoD Intramural and Extramural Animal Use by Service FY94

**TOTAL = 268,091**



**Figure V-2.** Total DoD Intramural Animal Use by Service FY94

**TOTAL = 332,592**



**Figure V-3.** Total DoD Extramural Animal Use by Service FY94

Percentages may not add up to 100% due to rounding of calculations

In FY94 the Army used 76.4% of the DoD total animal use, 62.2% of the total intramural animals and 87.9% of total extramural animals. The U.S. Army Medical Research and Materiel Command (USAMRMC) is the congressionally mandated Lead Agency for infectious disease and combat dentistry research and the DoD Executive Agent for medical chemical and medical biological defense and nutrition studies. The Command is responsible for greater than 85% of the DoD Medical Research, Development, Test and Evaluation programs. The Army's management of extramural animal-based research increased in FY94 as a direct result of increases in congressionally directed extramural research programs.

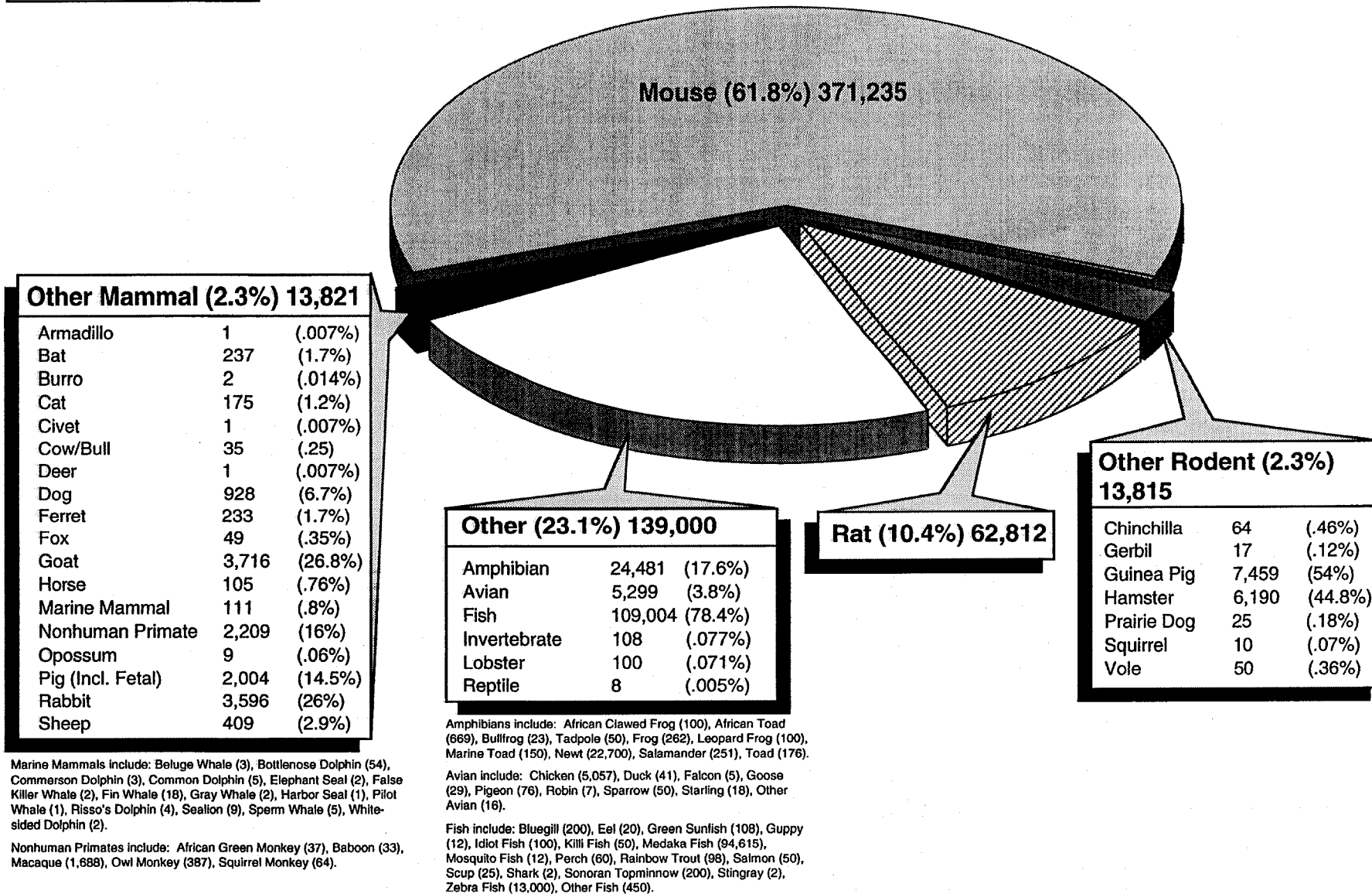
## **V.2.2 Animal Use by Species**

DoD animal use by species is presented in Figure V-4. Figures V-5 and V-6 represent the intramural and extramural animal use by species for FY94. The majority (~98%) of animals used by the DoD both intramurally and extramurally consisted of rodents, birds, amphibians and fish. There was an 8% increase in animal use from FY93 to FY94 which is largely a result of implementing alternatives that replace "higher" species with those that are lower on the phylogenetic scale. Fish usage was the single largest contributor to animal use increase: 34,328 fish were used in FY93, while 109,004 were used in FY94 (318% increase). Fish were used primarily in toxicity studies where they replace mammals, and thus represent the implementation of alternatives. Given the total FY94 increase of 46,983 animals, the 109,004 fish essentially replaced the use of 62,021 animals higher on the phylogenetic scale. From this perspective, the total usage of species higher on the phylogenetic scale actually *decreased* in FY94.

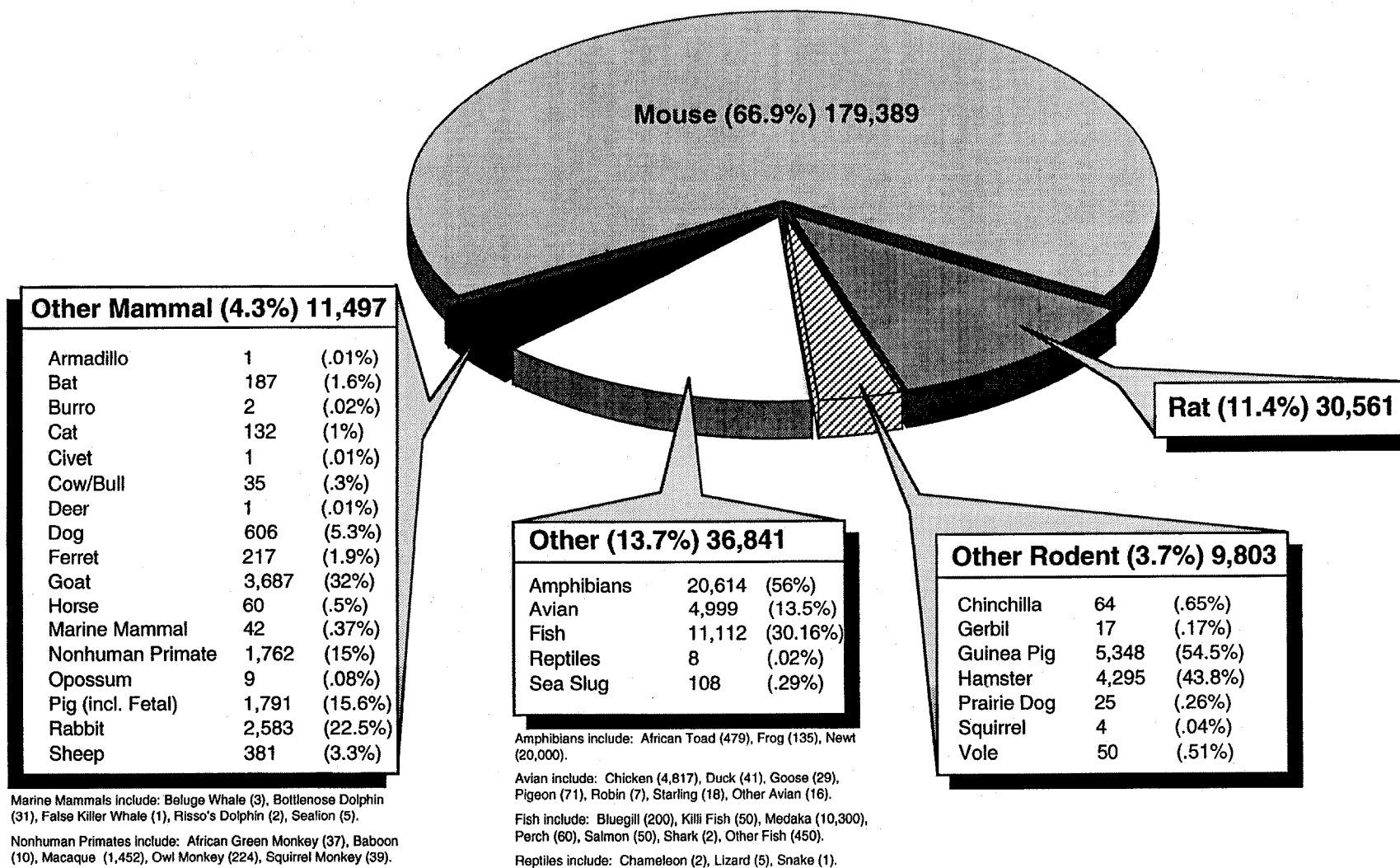
In addition to fish, significant increases were observed in the use of rats and mice. Due to the large numbers of mice and rats used, it is difficult to trace these increases to any particular study. However, in many instances, mice and rats are used as alternatives to higher mammals in vaccine and drug development studies.

The combined increases in fish, rats, and mice account for essentially all of the increased animal use in FY94. The observed increase in the use of these species was offset by decreases in the use of nonhuman primates, cats, rabbits, pigs, guinea pigs, hamsters, and ferrets.

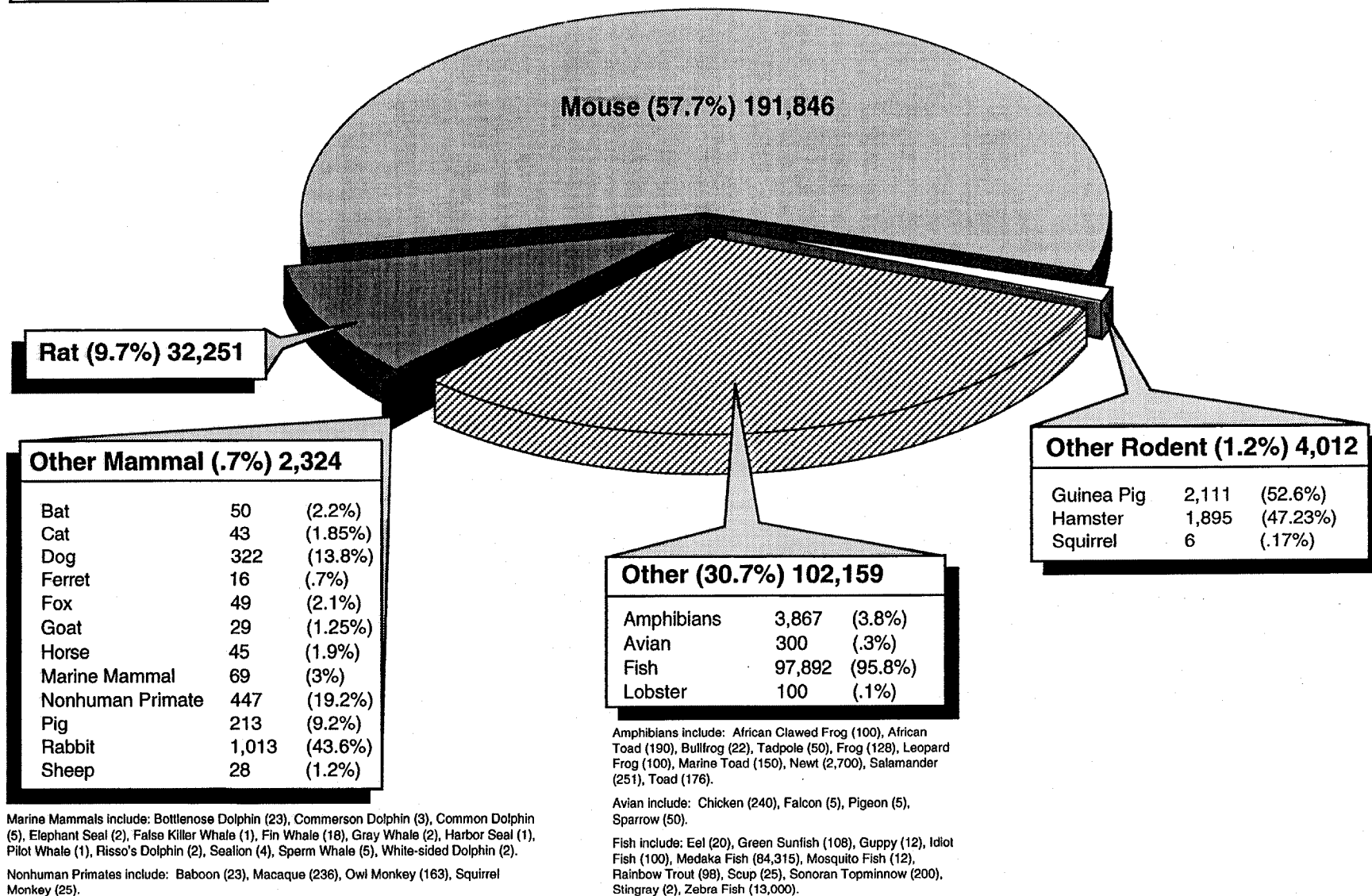
**TOTAL = 600,683**



V-7 **Figure V-4. Total DoD Intramural and Extramural Animal Use by Species FY94**

**TOTAL = 268,091**

**Figure V-5. Total DoD Intramural Animal Use by Species FY94**

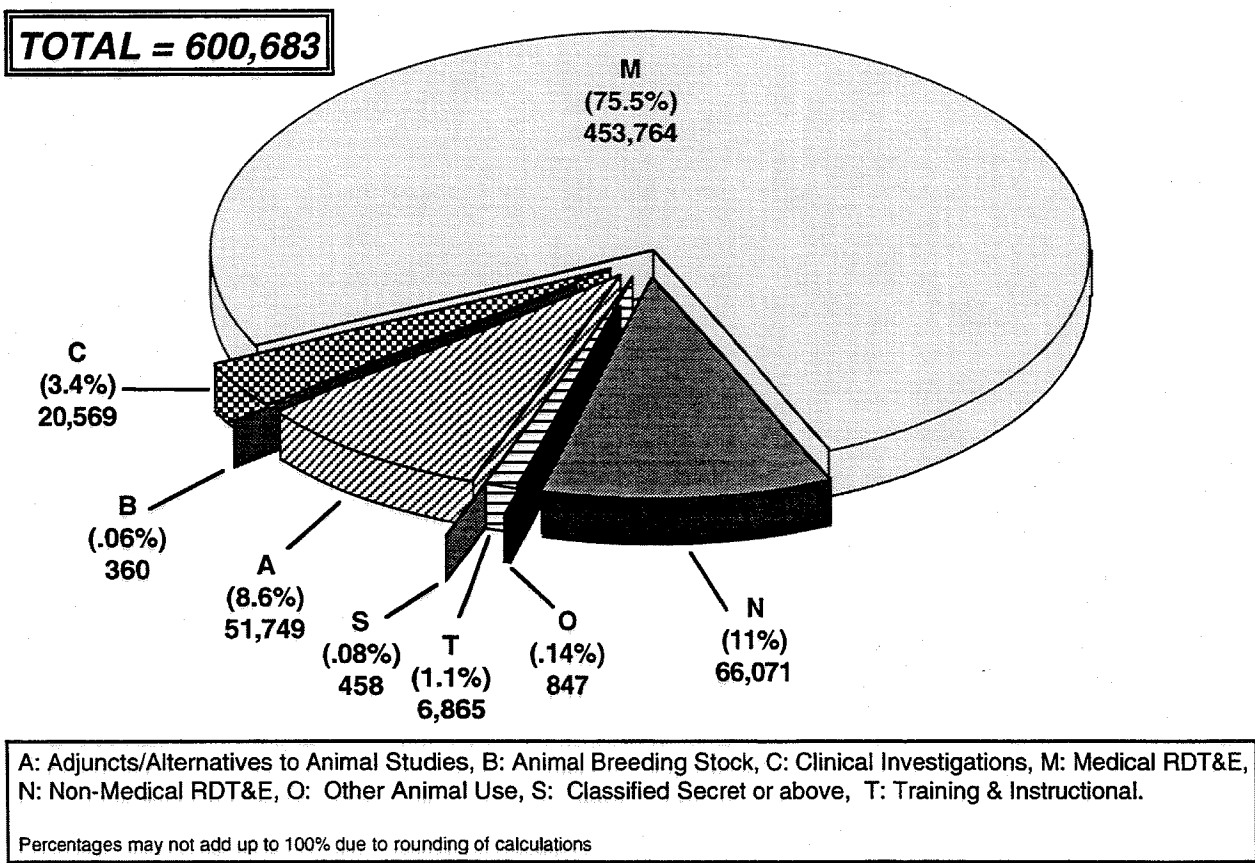
**TOTAL = 332,592**



**Figure V-6. Total DoD Extramural Animal Use by Species FY94**

### V.2.3 Animal Use by Category

Total animal use in the DoD by category is presented in Figure V-7, with the intramural and extramural breakouts in Figures V-8 and V-9, respectively. The DoD has a critical and challenging mission: to discover, design and develop military medical countermeasures against threats to the health and survivability of military personnel. In order to meet this mission, 75.5% of the animals used by the DoD in FY94 were in medical research. The majority (58%) of animals used in medical research were in the area of infectious diseases and consisted primarily of rodents (~96%) (Appendix L). The primary thrust of this research is the development of preventive measures against infectious disease through discovery, design, and development of prophylactic, therapeutic, and treatment drugs for relevant diseases. Non-medical RDT&E accounts for 11% of the animal use in FY94 consisting of primarily environmental research. Seventy-seven percent of the animals used in this area were fish. Research in the area of alternatives to the use of animals was 8.6% of the total animal usage for FY94 and utilized primarily fish (99%). This illustrates the Department's continuing initiatives to promote research to develop alternatives to reduce, replace and refine the use of animals in DoD research. No animals were used for offensive weapons testing during FY94.



**Figure V-7.** Total DoD Intramural and Extramural Animal Use by Category FY94

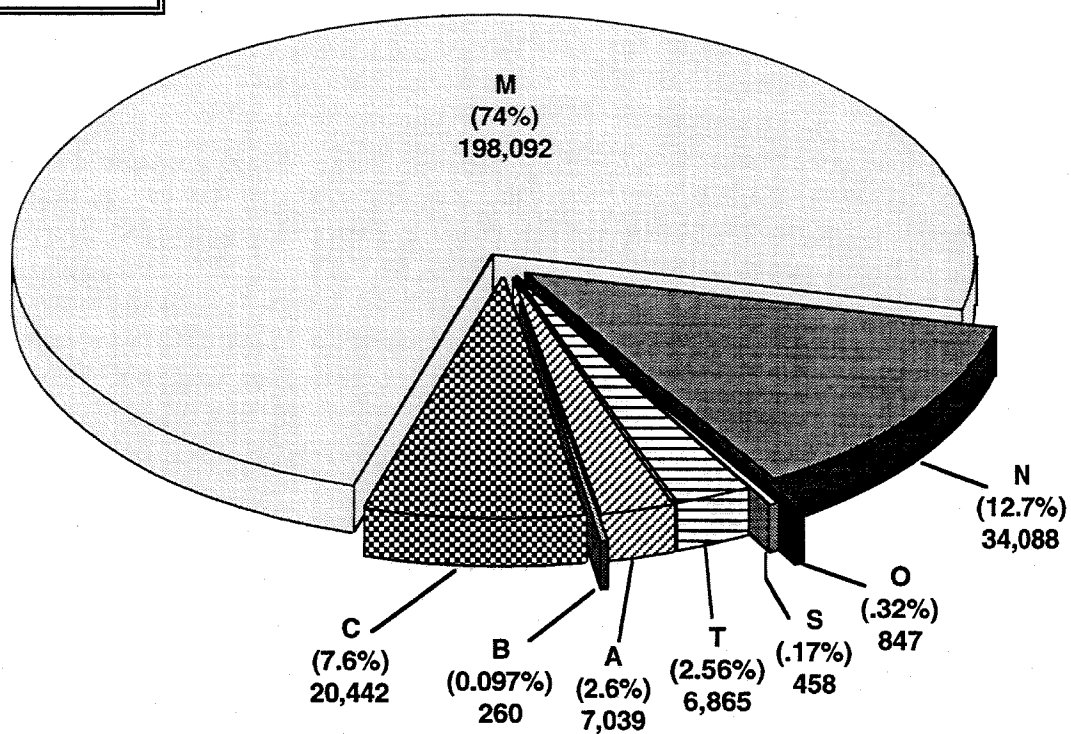
**TOTAL = 268,091**

Figure V-8. Total DoD Intramural Animal Use by Category FY94

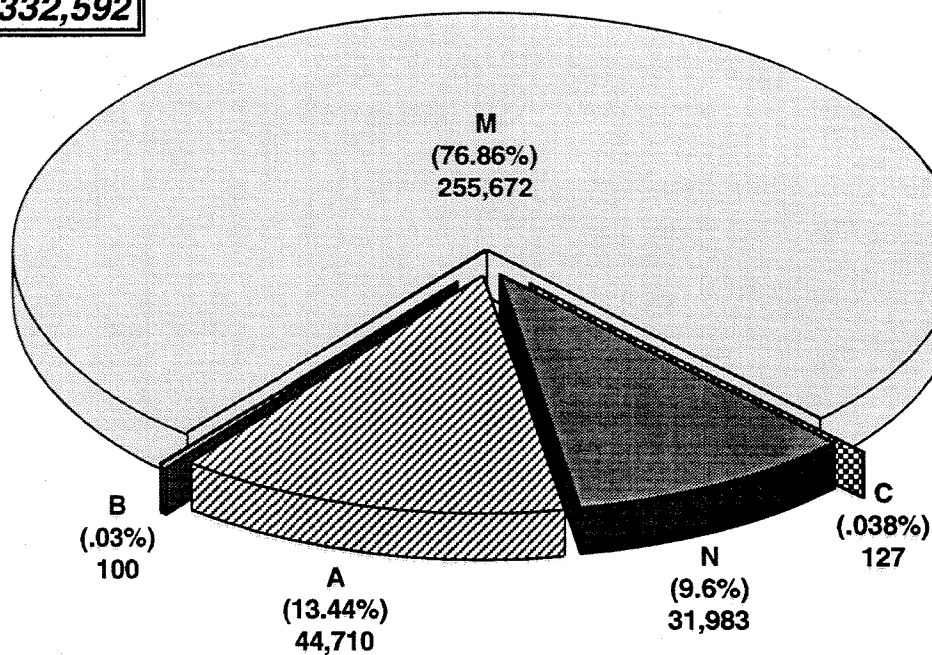
**TOTAL = 332,592**

Figure V-9. Total DoD Extramural Animal Use by Category FY94

A: Adjuncts/Alternatives to Animal Studies, B: Animal Breeding Stock, C: Clinical Investigations, M: Medical RDT&E, N: Non-Medical RDT&E, O: Other Animal Use, S: Classified Secret or above, T: Training & Instructional.

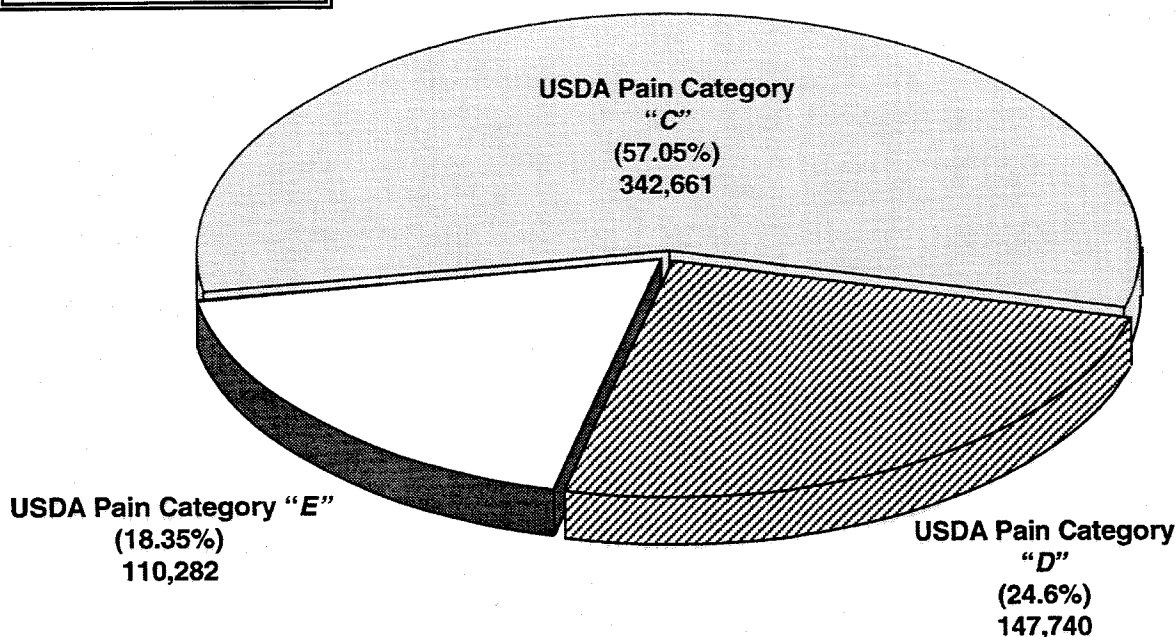
Percentages may not add up to 100% due to rounding of calculations



## V.2.4 Animal Use by USDA Pain Category

Total animal use in the DoD by USDA pain category is presented in Figure V-10, with the intramural and extramural breakouts in Figures V-11 and V-12, respectively. Most research (82%) in the DoD was not painful to the animals involved. In the majority of the cases (57%), the animals were not exposed to or involved in any painful procedures. In 25% of the cases, animals were given anesthesia or pain-relieving drugs during procedures that could have involved some pain or distress to the animals. In 18% of the animals, anesthetics or analgesics were not used because they would have interfered with the results of experiments. Most (97%) of the animals used in painful experiments (where the drugs would have interfered with the results) were rats and mice. Greater than 96% of these rodents were used in research categories M2, M3, M4 and M7, as defined in Table V-1. These categories include research on infectious diseases such as malaria, leishmaniasis, HIV and all medical research in support of defense against nuclear, biological and chemical threats. A large fraction of these studies is driven by federal requirements, particularly those of the Food and Drug Administration (FDA).

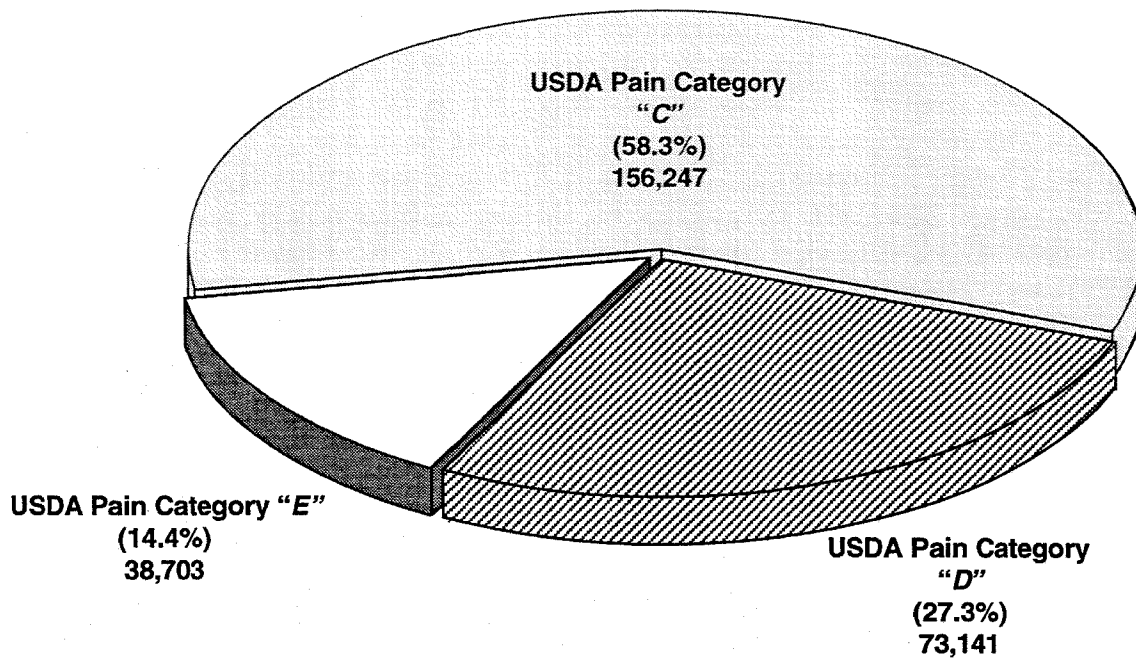
**TOTAL = 600,683**



Percentages may not add up to 100% due to rounding of calculations

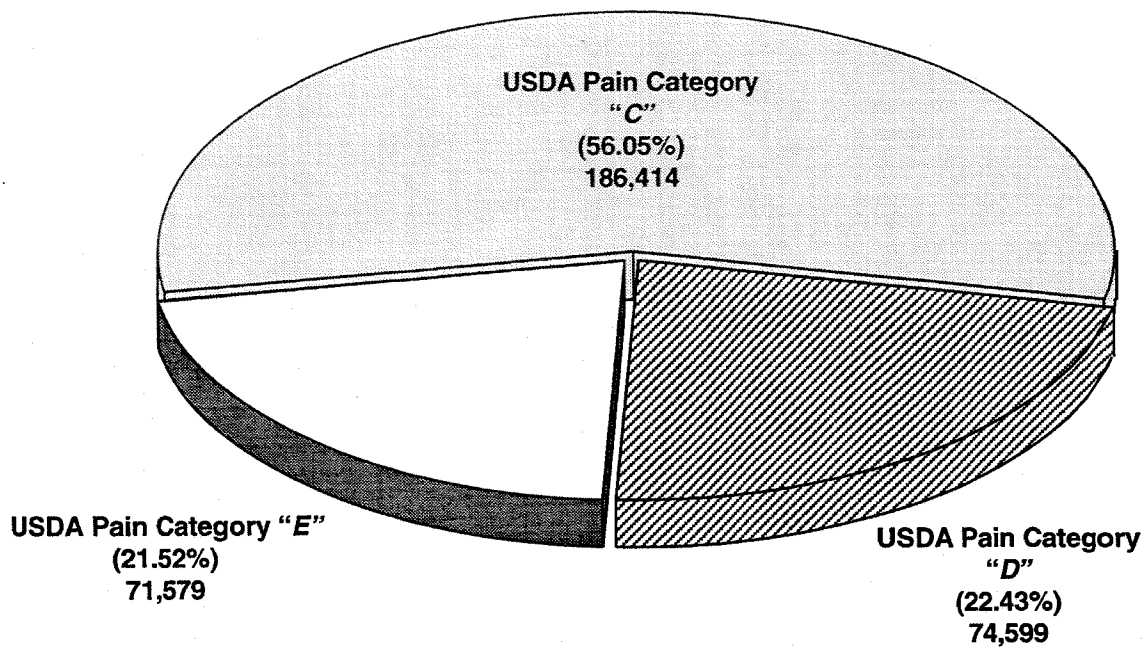
**Figure V-10.** Total DoD Intramural and Extramural Animal Use by USDA Pain Category FY94

**TOTAL = 268,091**



**Figure V-11.** Total DoD Intramural Animal Use by USDA Pain Category FY94

**TOTAL = 332,592**



**Figure V-12.** Total DoD Extramural Animal Use by USDA Pain Category FY94

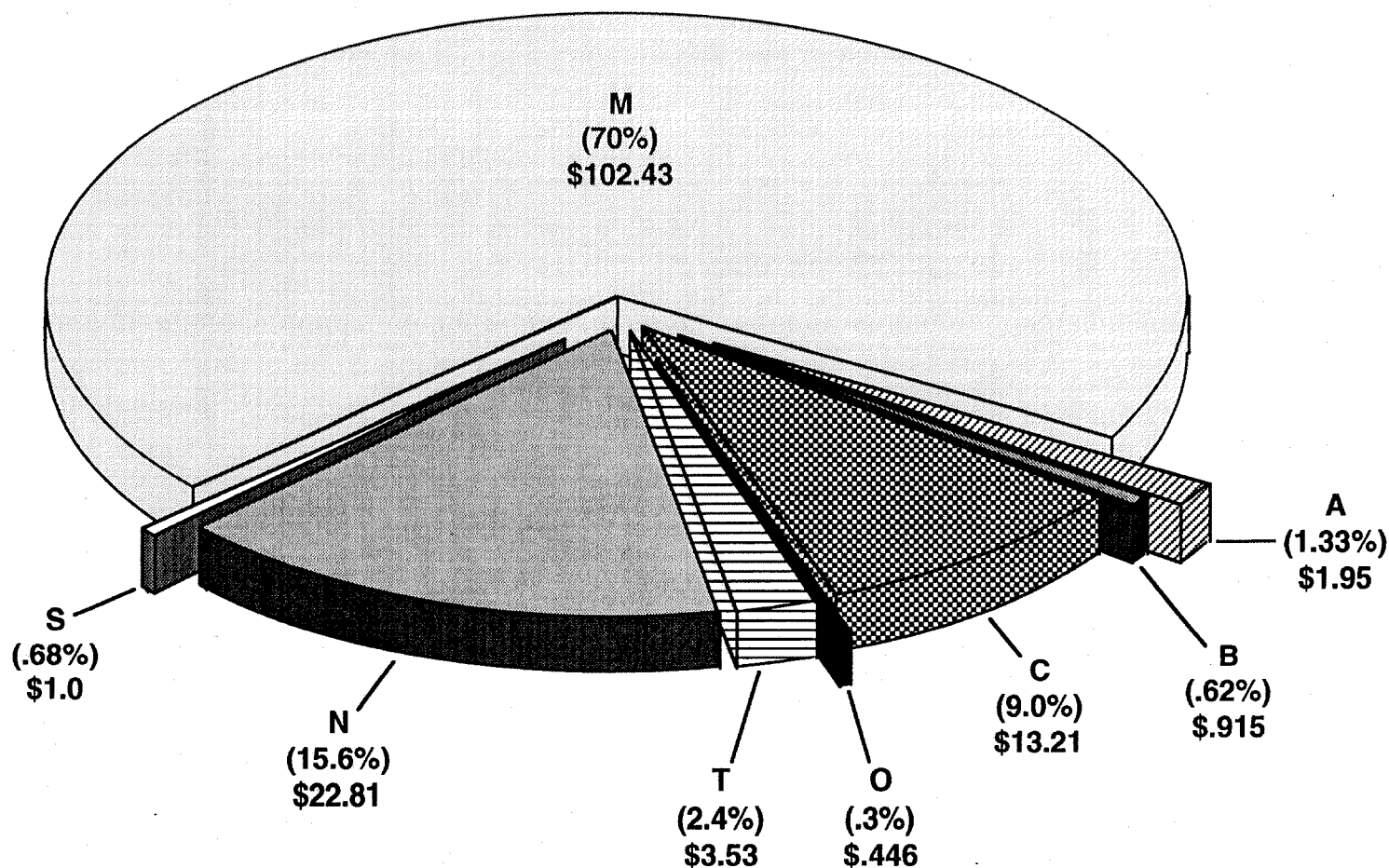
Percentages may not add up to 100% due to rounding of calculations

### **V.2.5 Animal Use Funding by Category**

Total animal use funding in DoD by category is presented in Figure V-13. Data for intramural DoD, intramural non-DoD, and extramural funding by research category are presented in Table V-3. Research and development funds are identified within the Department as Major Force Program 6, or P-6 funds. Training and education activities utilizing animals are supported with Major Force Program 8, or P-8 funds. These funds are also presented for those activities engaged in training and education. The DoD-funded \$146.3M on animal-based research in 1994. Medical research accounted for approximately 70% of the total animal use funding. This correlates well with both the number of animals used in medical research and the DoD medical research mission. Non-medical RDT&E represented 15.6% of the total animal use funding. There were no animals used for offensive weapons testing research and there were no funds spent on animals in this research category (N3). Almost \$2M were spent on developing alternatives to animal use during 1994, again demonstrating the Department's commitment to the reduction, replacement, refinement and responsibility of the use of animals in research. While there were no adjunct protocols focusing specifically on animal husbandry and care, there were several actions in this area. As an example, Walter Reed Army Institute of Research has established a policy (WRAIR Policy Letter 93-27, Appendix M) which mandates consideration for environmental enrichment for research animals. This policy allows for flexibility and creativity for improving conditions of laboratory animals.

**TOTAL = \$146,332**

**(\$M)**



A: Adjuncts/Alternatives to Animal Studies, B: Animal Breeding Stock, C: Clinical Investigations, M: Medical RDT&E, N: Non-Medical RDT&E, O: Other Animal Use, S: Classified Secret or above, T: Training & Instructional.

Percentages may not add up to 100% due to rounding of calculations

**Figure V-13. Total DoD Intramural and Extramural Animal Cost by Category FY94**

**Table V-3. Funding of Animal Use Programs by Category (\$K)**

CATEGORY	IM DoD	IM Non DoD	IM Total	EM DoD	Grand Total
Military Dentistry (M1)	504	23	527	137	664
Infectious Diseases (M2)	14,798	285	15,083	5,012	20,095
Medical Chemical Defense (M3)	10,713	0	10,713	8,653	19,366
Medical Biological Defense (M4)	13,252	0	13,252	3,845	17,097
Human Systems Technology (M5)	3,604	0	3,604	8,322	11,926
Combat Casualty Care (M6)	3,172	0	3,172	8,724	11,896
Ionizing Radiation (M7)	3,711	463	4,174	660	4,834
Other Medical RDT&E (M8)	1,288	0	1,288	15,267	16,555
<b>MEDICAL TOTAL</b>	<b>51,042</b>	<b>771</b>	<b>51,813</b>	<b>50,620</b>	<b>102,433</b>
Physical Protection (N1)	328	0	328	0	328
Physical Detection (N2)	2,855	0	2,855	1,501	4,356
Offensive Weapons Testing (N3)	0	0	0	0	0
Other Non-Medical RDT&E (N4)	8,765	277	9,042	9,090	18,132
<b>NON-MEDICAL TOTAL</b>	<b>11,948</b>	<b>277</b>	<b>12,225</b>	<b>10,591</b>	<b>22,816</b>
Clinical Medicine (C1)	1,169	9,430	10,599	44	10,643
Clinical Surgery (C2)	578	219	797	3	800
Other Clinical Investigations (C3)	1,738	37	1,775	0	1,775
<b>CLINICAL INVESTIGATIONS TOTAL</b>	<b>3,485</b>	<b>9,686</b>	<b>13,171</b>	<b>47</b>	<b>13,218</b>
Training, Education, Instruction (T1)	3,390	0	3,390	0	3,390
Other Training/Instruction (T2)	149	0	149	0	149
<b>TRNG. &amp; INSTRUCTIONAL TOTAL</b>	<b>3,539</b>	<b>0</b>	<b>3,539</b>	<b>0</b>	<b>3,539</b>
Adjuncts to Animal Use Research (A1)	0	0	0	0	0
Alternatives to Animal Invest. (A2)	1,079	0	1,079	818	1,897
Other Alternatives/Adjuncts (A3)	60	0	60	0	60
<b>ALTERNATIVES TOTAL</b>	<b>1,139</b>	<b>0</b>	<b>1,139</b>	<b>818</b>	<b>1,957</b>
Classified Secret or Above (S)	1,006	0	1,006	0	1,006
Breeding Stock (B)	878	0	878	38	916
Other Animal Use Purposes (O)	294	153	447	0	447
<b>SECRET/BREEDING/OTHER TOTAL</b>	<b>2,178</b>	<b>153</b>	<b>2,331</b>	<b>38</b>	<b>2,369</b>
<b>GRAND TOTAL</b>	<b>73,331</b>	<b>10,887</b>	<b>84,218</b>	<b>62,114</b>	<b>146,332</b>

## DoD INITIATIVES TO PROMOTE ALTERNATIVE METHODS THAT REPLACE, REDUCE AND REFINE THE USE OF ANIMALS

## Section **VI**

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This section responds to the Committee's continuing direction that the Secretary of Defense "establish aggressive programs to replace, reduce and refine current uses of animals" (H.R. 4301, 1995). Alternatives, as articulated in *The Principles of Humane Experimental Technique* (Russell and Burch, 1959), are defined as methods that **Replace, Reduce and Refine** the use of animals. In addition to these *Three Rs*, the Department of Defense (DoD) advocates a fourth *R*, "Responsibility," for implementing these alternative methods.

Department policy with regard to animal alternatives is promulgated in DoD Directive 3216.1 which directs that "it is DoD policy that...alternatives to animal species should be used if they produce scientifically satisfactory results...." This policy is implemented in the Joint Service Regulation on the Use of Animals in DoD Programs, which delegates responsibility to the local commander for utilization of alternatives to animals.

To illustrate the Department's initiatives to promote these *Four Rs*, a description of such initiatives within DoD's research laboratories and medical treatment centers is provided. The following list is not all inclusive, as the number of specific examples of implementing alternative methods that can be documented for DoD's research projects is large. Rather, it illustrates the scope, diversity, and spirit of DoD's *Four Rs* initiatives. This section will demonstrate a broad-based movement, where feasible, towards the use of biotechnology and other innovative alternatives to replace and reduce animal use as well as refinement in methods used in essential animal studies.

### VI.1 RESPONSIBILITY

The DoD has established a variety of initiatives and targeted programs that are currently in place to promote alternative methods that will replace, reduce and refine the use of animals. These programs are designed to target individual and institutional awareness by providing educational opportunities, professional training and fiscal resources towards implementing the *Four Rs* approach to minimizing animal use.

### **VI.1.1 Science and Technology Emphasis on Alternatives to Animal Subjects of Research**

An example of the Department's direction on seeking alternatives to animal use is the fiscal year (FY) 1993 Army Science and Technology Objective (STO) entitled *Reducing Reliance on Human and Animal Subjects of Research*. The specific task to "Develop refinement, reduction and replacement strategies for projects currently reliant on the use of animals" supports this STO and is designed to provide a positive mechanism for researchers to explore and implement alternatives to the use of animals. This provides both the impetus for alternatives implementation, as well as a mechanism for funding such research. In FY94, this Army STO was revised and strengthened. The title for this objective for FY94 is *Reducing Reliance on Animals for Research and Improving Experimental Conditions Using Animals* (ASTMP, FY94). The U.S. Army Medical Research and Materiel Command (USAMRMC) budgets \$600,000 per year for this objective, which is available to support alternatives to animal use research in all three services. In addition, the USAMRMC funded a four year, \$604,000 contract (91-C-1049) to develop and validate the Frog Embryo Teratogenesis Assay for detecting environmental toxicants and teratogens. This system is an alternative to the use of mature animals in this type of research.

Army STOs provide guidance, means, and high visibility to major Army technology initiatives. The Department of the Army, in coordination with the Director of Defense Research and Engineering (DDR&E), Office of the Secretary of Defense (OSD), publishes the *Army Science and Technology Master Plan* (ASTMP) as guidance to Army laboratories and research, development and engineering centers and to non-Army organizations supporting the Army science and technology (S&T) base.

### **VI.1.2 Conferences and Workshops on Alternatives to Animal Use**

The DoD promotes responsibility for alternatives to animal use by sponsoring formal education training programs and major meetings and conferences on the subject. In 1990, an important conference on alternatives to animal use, "DoD Initiatives in Alternatives to Animal Testing," was held at Aberdeen Proving Ground. This was followed by a three-day symposium in 1992 entitled "Current Concepts and Approaches on Animal Test Alternatives" with 35 scientific platform sessions and 22 scientific poster presentations. This international symposium was attended by nearly 300 military and civilian scientists from four countries. The symposium was praised as a success by Dr. Martin Stephens of the Humane Society of the United States (Appendix N). Proceedings of the 1992 symposium were published in September 1993 and are available through the Defense Technical Information Center. In addition, in 1994 a book edited by Dr. Harry Salem entitled "Animal Test Alternatives" was published by Marcel Dekker, Inc., which included chapters prepared by most of the presenters at this symposium (Appendix O). The Department's continuing commitment to promoting responsibility for alternatives to animal use, even in an environment of constrained resources, is reflected by

another such conference held on 24-26 May 1994, at Aberdeen Proving Ground entitled "Alternatives in the Assessment of Toxicity: Theory and Practice." This international conference with 26 scientific platform sessions, including one by Dr. Martin Stephens of the Humane Society of the United States (Appendix P), and 45 scientific poster presentations was attended by over 330 military and civilian scientists from seven countries. The proceedings and a monograph based on this successful symposium are in preparation. In addition, DoD is represented on the Interagency Regulatory Alternatives Group which has planned and presented a "Workshop on Updating Eye Irritation Test Methods" in 1991 and is currently planning another workshop on Dermal Testing. The National Institute of Environmental Health Sciences has established the Interagency Coordinating Committee on the Validation of Alternative Methods in response to the Revitalization Act of 1993, which also has DoD representation. Presentations have also been made on alternatives to the Board of Scientific Councilors of the National Toxicology Program of the National Institute of Environmental Health Sciences (NTP-NIEHS), Board of Scientific Councilors of the Food and Drug Administration and Cancer Etiology Group at the National Cancer Institute.

#### **VI.1.3 National Research Council, Institute of Laboratory Animal Resources, Educational Programs**

The DoD's priority and continuing commitment to promoting individual and institutional responsibility for alternatives to animal use are reflected in continuing financial support of the Institute of Laboratory Animal Resources (ILAR) educational program of the National Research Council. The principal thrust of the ILAR grant is development of institutional training materials, educational courses and publications in support of the Department's laboratory animal care and use programs. This ILAR information is used in various military research facilities as an important adjunct to existing investigator training and technical education programs on animal care and use. The ILAR information and programs have generated strong animal alternative provisions for military-specific research. The Department previously funded a five-year ILAR grant (DAMD 17-87-G-7021) for this program and is currently in the third year of another five-year ILAR grant (DAMD 17-93-J-3016) committing diminishing research funds to maintain this important collaboration. Annual funding for this DoD-sponsored ILAR program is in excess of \$100,000. In addition, National Research Council fellowships for conducting research in alternatives to animals are available at the U.S. Army Edgewood Research, Development, and Engineering Center, Aberdeen Proving Ground, Maryland (Appendix Q).

#### **VI.1.4 Institutional Animal Care and Use Committee Emphasis**

Title 9 (Animals and Animal Products), Subchapter A (Animal Welfare), Parts 1-4 of the Code of Federal Regulations has specific provisions for addressing the issue of alternatives during the research animal protocol review process. The DoD has been a leader in forming lawfully constituted and functioning Institutional Animal Care and Use Committees (IACUCs) at its biomedical research facilities.



Accordingly, DoD IACUCs consider alternatives to the proposed use of animals as an important review consideration. The IACUC will use the DoD Standard IACUC Protocol Format for animal use proposals, which requires that non-animal alternatives be considered. It states that "No study using animals should be considered prior to the elimination of all reasonable possibilities that the question might be adequately answered using other than animal means." Investigators must provide information on the animal model being proposed and justification for the selected species. The Standard Protocol Format states that "investigators should use the least sentient species that will permit the attainment of research objectives." In addition, the investigators are required to provide a short description of the features of the proposal that may qualify the study as one that refines, reduces or replaces the use of animals. The DoD 1995 Policy letter requires that extramural contractor proposals utilizing animals in research, testing or training include all the information contained in the DoD Standard Protocol Format, thereby requiring them to also provide the alternatives information.

#### **VI.1.5 Veterinary Staff Expertise and Assistance Visits**

The major biomedical research commands of the Military Departments each have credentialed laboratory animal medicine (LAM) veterinarians serving in key staff positions. Approximately 5% of the board-certified specialists of the American College of Laboratory Animal Medicine (ACLAM) currently serve in the DoD. In addition to being advisors to commanders on issues related to animal welfare and alternatives to animal use, these veterinarians provide oversight and structure to the command's animal care and use programs. These officers also make periodic staff assistance visits to subordinate facilities that use animals and evaluate each laboratory animal care and use program. Consideration of the use of alternatives is reviewed on these staff assistance visits. Another important responsibility of the LAM veterinarian is to review extramural animal use protocols, ensuring that alternatives to animal use and personnel training issues have been addressed.

#### **VI.1.6 Professional Veterinary Training in LAM**

The presence of specialty trained, veterinary, LAM expertise in biomedical research institutions strongly correlates to effective animal use alternatives programs. This is especially true in the critical area of refinements. The DoD has long been a leader in training veterinarians in the field of LAM, the biomedical and veterinary specialty most closely associated with laboratory animal welfare and laboratory animal care and use programs. Many of the nationally prominent leaders of several laboratory animal associations were formally trained in, or closely associated with, DoD LAM training programs. Examples are the President and several past presidents of ACLAM, the President-elect and several past presidents of the American Association of Laboratory Animal Science (AALAS), and several past presidents and the current Secretary-Treasurer of the American Society of Laboratory Animal Practitioners. This traditional DoD strength in LAM expertise strongly enhances both animal care and use and animal alternatives programs.

Approximately 25% of all ACLAM boarded specialists in the U.S. received some or all of their LAM training in DoD LAM training programs.

#### **VI.1.7 AALAS Technician and Laboratory Animal Science Training**

There are a number of DoD research facilities that sponsor formal training programs leading to certification of animal care and research personnel as AALAS laboratory animal technicians. This specialized training is offered to both government and non-government animal technicians. It is an important mechanism for ensuring highly qualified animal care and research technicians in Defense laboratories. Individual DoD institutions have sponsored formal seminars for research personnel where experts from the National Agricultural Library explain in detail the resources available for exploring various animal alternatives in the laboratory. The Walter Reed Army Institute of Research (WRAIR) sponsors laboratory animal workshops that provide comprehensive technical training available to all DoD personnel on animal use and related issues. Improving the technical expertise of laboratory animal technicians and investigators is a significant refinement element for the use of animals in the laboratory. These workshops are available to all DoD and National Institutes of Health laboratories. As an example, the workshop on the use of rodents is offered 14 times per year. In addition, WRAIR offers quarterly a workshop on ethical and administrative issues relating to animal use. The AALAS technicians' course curriculum and the WRAIR workshop curriculum include formal training and information on alternatives to animal use.

### **VI.2 DoD INITIATIVES TO REPLACE, REDUCE AND REFINES THE USE OF ANIMALS**

The following specific examples are a representative listing of alternative methodologies practiced in DoD facilities. They are categorized as Replacement, Reduction, and Refinement initiatives. Because of the multifaceted aspects of many of these examples, some logically belong in more than one category. Examples with an asterisk (\*) indicate an alternative first reported in FY94 by a DoD facility or extramural contractor. Examples with a bullet (•) indicate an alternative reported in FY93 and FY94.

#### **VI.2.1 Replacement**

The replacement alternative addresses supplanting animal use with non-living systems, analytical assays, cell-culture systems, and with animals that are lower on the phylogenetic scale. Additionally, human subjects are used when experimental drugs and other procedures progress to human trials. Such trials are conducted in accordance with Title 32, U.S. Code of Federal Regulations, Section 219, "Protection of Human Subjects in DoD-Sponsored Research."

#### VI.2.1.A Replacement Using Biochemical or Physical Methods

- Membrane feeding systems have been developed that replace the need to feed some types of blood-feeding flies and mosquitos on rodent hosts.
- Development of Polymerase Chain Reaction (PCR) and Mammalian Cell Selection Assays for short-term genetic toxicity testing replaces animal use in carcinogenesis and mutagenesis studies.
- Efforts are ongoing to develop a PCR assay for Q-fever that could eliminate the need for the use of a mouse bioassay.
- \* Use of PCR for assessment of viral infections.
- Quantitating bacterial endotoxin with an *in vitro* Limulus Amebocyte test replaces *in vivo* pyrogen testing in rabbits.
- Use of predictive anthropomorphic dummies and manikins, e.g., ADAM (ejection seat reactive live load manikin) and AIRMAN (a fragment capture live fire manikin) has replaced the use of animals in these studies.

#### VI.2.1.B Replacement Using Computer Simulations

- Computer models to replace rhesus monkeys and baboons for toxicological studies are being developed.
- Development of computational models of dolphin echolocation (sonar) for inclusion in the development of hardware systems will replace use of animals as object detectors.
- Development for Special Forces medical training personnel of advanced computer technology using Virtual Reality, Holographic Imaging, and Telepresence Surgery techniques may replace the use of animals in Special Forces surgical training.
- Computer models are being developed for predicting carcinogenesis induced by ionizing radiation replacing the need to use animals.
- \* A computer model for predicting the transfer of toxic chemicals across the intestinal mucosa and into the blood stream is in development.

#### VI.2.1.C Replacement Using *in vitro* Cell Culture

- *In vitro* cell culture methods have been developed for passage of Hepatitis E virus eliminating use of animals for virus propagation.

- Development of a macrophage cell line to replace animals in evaluation of cytotoxicity and genotoxicity of respirable particles is in progress.
- Development of a fish liver cell culture model for evaluating metabolism of Xenobiotic compounds replaces the use of mammalian animal models.
- \* Tissue culture using human gingival fibroblasts replaced the need to use rats to study the effects of Transforming Growth Factor-Beta (TGF- $\beta$ ) on wound healing.
- \* Cell cultures are being evaluated to replace mice as a host assay for detecting and identifying arthropod-borne viruses.
- \* Use of a rat cell line obtained from the American Type Culture Collection to study Calcium Channel Blockers and Angiotensin Converting Enzyme Inhibitors eliminates the use of pigs.
- \* Established cell lines from American Type Culture Collection are used in place of mice to test the effects of antibiotics on cell proliferation and inhibition of DNA synthesis as well as to test the effects of anti-progesterone chemicals on proliferation and/or inhibition and tumor cell death.
- \* Established cell line of macrophages from American Type Culture Collection to study the alteration of macrophage chemotactic response by oxygen replaces the use of mice and rats.
- \* Development of an *in vitro* hepatotoxicity screen to rank order chemicals for their ability to damage the liver will replace the use of mice and rats.
- \* Use of human mononuclear cells analyzed by flow cytometry to determine expression of CD69 after staphylococcal enterotoxin B (SEB) treatment, may replace the use of mouse spleen cells.
- \* Cell and organ cultures to replace the rabbit for mucin-type glycoprotein in malignant breast tissue studies.
- \* Cell and organ cultures to replace the rat in regulated mucin gene expression in airway injury studies.
- \* Use of human and animal peripheral blood lymphocytes with flow cytometry to assess cytotoxicity and DNA alterations induced by sulfur mustard and its monofunctional analogue chloroethyl-ethyl sulfide replaces the use of hairless guinea pig and weanling domestic swine.

- \* Human cortical cell lines (HCN-1A) were used instead of rats to determine specific characteristics of sodium channels, in an effort to confirm their usefulness in studying toxins that produce their effect by sodium channel blocking.
- \* The HeLa Cell, a human epithelial tumor line, has been established as a useful proliferating cell model in sulphur mustard studies, replacing the use of hairless guinea pigs and weanling domestic swine.
- \* A terminal deoxynucleotide transferase assay was developed to measure the presence of DNA single strand breaks following sulfur mustard exposure of peripheral blood lymphocytes and human epidermal keratinocytes, replacing the use of hairless guinea pigs and weanling domestic swine.
- \* Human epidermal keratinocytes cultures are used as a model system to study the change in poly (ADP-ribose) polymerase (PADPRP) activity levels following sulfur mustard exposure, replacing the use of hairless guinea pigs and weanling domestic swine.
- \* Studies with neuroglioma cells in culture (NG108-15 cells) suggest that they are acceptable for validation of the poly (ADP-ribose) polymerase (PADPRP) assay and for preliminary concentration dose response curves of sulfur mustard-induced cytotoxicity and PADPRP activity, replacing the use of hairless guinea pigs, rats, mice, and guinea pigs.
- \* Cells are exposed to dilute liquid sulfur mustard. At selected intervals post-exposure, samples are prepared for either biochemical or ultrastructural analyses. Analysis includes the application of specific probes (e.g., antibodies) or gel electrophoresis and electron microscopic autoradiography. Once identified, specific molecular targets are developed for use as biological markers in antivesicant drug assessment. These techniques will replace the use of hairless guinea pigs and guinea pigs.
- \* Living "TESTSKIN" (the commercial human skin equivalent) cellular models are used to elucidate the biochemical mechanisms responsible for sulfur mustard-induced pathology, replacing the use of hairless guinea pigs and weanling domestic swine. As the mechanisms are defined, studies of therapeutic intervention are evaluated for protection against sulfur mustard-induced pathology.
- \* A contract with the Cooperative Human Tissue Network provides human skin biopsies that replace the use of hairless guinea pigs and weanling domestic swine. Techniques for explant culture were developed and the specimens evaluated for histologic integrity over the first 5 days following receipt.

- \* Transendothelial electrical resistance and ultrastructure of cultured bovine pulmonary endothelial cells are determined after direct exposure to three edemagenic gases: phosgene, perfluoroisobutylene, and bis (trifluoromethyl) disulfide. Membrane electrical resistance is a sensitive method of determining tissue integrity and can be used to assess changes in cell-to-cell interactions that affect permeability of the endothelial barrier. These techniques replace the use of rats, guinea pigs, and sheep.
- \* Clonal neurosecretory cells of adrenal chromaffin or clonal pheochromocytoma origin are individually injected with botulinum toxin or the purified light chain of botulinum toxoid. Patch clamp recordings are used to measure capacitance changes associated with fusion of neurosecretory vesicles with the plasma membrane. Detailed examinations of membrane events during vesicle fusion are performed in the presence and absence of botulinum toxin. These techniques replace the use of mice, rats, and guinea pigs.
- \* Clonal neurosecretory cells of adrenal chromaffin or clonal pheochromocytoma origin are transfected with antisense oligonucleotides to suppress protein production from specific mRNA's. Secretion of the vesicle contents in response to potassium stimulation is then measured to assess the importance of the suppressed protein to synaptic transmission. These techniques replace the use of mice, rats, and guinea pigs.
- Study of the effects of growth factors on human fibroblasts is being conducted in cell culture media replacing the dogs and pigs utilized in previous studies.
- Development of a cell culture system to pass human breast cancer cells eliminates the need for initially passing these cells in a nude mouse model.
- Use of immortalized tissue culture systems or isolated lobster neuronal cells to investigate radiation effects and free radical damage to the nervous system at the molecular level are used to replace similar protocols using rats and guinea pigs.
- Wound-healing studies on space shuttle flights STS-45, 55 and 56 used a cell culture flight module instead of live rats.
- Development of human skin cell and animal processing plant skin models for assessing cellular mediator and tissue damage from environmental heat has replaced mammalian laboratory animal use.

#### **VI.2.1.D Replacement with Non-Mammalian Species and Species Lower in the Phylogenetic Scale**

- Development of an aquatic bioassay using the medaka fish (*Oryzias latipes*) to assess human carcinogenic health risks replaces laboratory animal use for tumor immunodiagnosis.
- \* Rats and swine may replace cynomolgus monkeys as an alternative model for hepatitis E.
- \* Pigs used in emergency room and surgical resident training; and hamsters, rabbits, pigs and rats in veterinary proficiency training to replace dogs.
- \* Ferrets used in pediatric advanced life support courses and endotracheal intubation exercises to replace cats.
- \* Development of genotoxicity model using fish as an alternative to the conventional rodent model.
- \* Cardiopulmonary measurements previously conducted in monkeys and guinea pigs are now carried out in free-moving unrestrained rats.

#### **VI.2.1.E Replacement with Human Tissue, or Volunteers as Protocols Progress to Human Trials**

- Many procedures including conjunctival impression cytology, salt and water balance and intestinal permeability, neuroendocrine assessment, nutritional support, testing of topical treatments and studies of *in vitro* activated keratinocytes in autografts in thermal injury research were previously performed in animals but have now progressed to human use protocols, eliminating the use of animals.
- Biomechanical analysis of the strength of plate fixation devices for long bone fracture repair is being performed with human cadaver bones and metal substitutes thereby replacing animal studies.

#### **VI.2.1.F Replacement with Discarded Tissue from Other Laboratories or Food Processing Plants**

- Pigs feet obtained from a local plant are used for teaching surgical suturing procedures, replacing the need for use of live animals.
- Sheep parts purchased from a processing plant are used to train dentists on periodontal surgical procedures replacing the use of live animals for training.

- Ocular researchers are using eyes purchased from local cattle processing plants for studies instead of live rabbits.
- Training programs for urology residents utilizing lasers for bladder treatments are initially performed with pig bladders purchased from a processing plant. This reduces the number of animals used for surgical training.
- Evaluation of suture patterns and angioplasty balloons on vein graft anastomosis on pigs used for surgical procedure training. Sharing of animals reduces the total number of animals used.

## **VI.2.2 Reduction**

Decreasing the numbers of animals used through the use of statistical or innovative design strategies, while preserving the scientific integrity of the biological model, is a major emphasis of the reduction alternative to animal use.

### **VI.2.2.A Reduction by Use of Alternative Screening Methods to Study Efficacy in Biological Testing**

- Development of a Quantitative Luminescence Imaging System for screening radiofrequency radiation biological effects in cells reduces the number of laboratory animals needed.
- Establishment of a tissue culture system to evaluate initial exposure levels of toxic substances, such as ammonia, or nitrogen and sulfur oxides, in lung and throat secretions reduces the use of animals in subsequent therapy studies.
- Development of an *in vitro* test using human peripheral blood could determine the effectiveness of toxoid in a SEB vaccine and measure the effectiveness of potential treatments to SEB poisoning. If validated, this would significantly reduce the animals used in SEB research.
- Use of bacteria, algae, crustaceans, earthworms, flatworms, and a toxicity estimation software program functions as a screening mechanism in toxicity testing, highlighting those chemicals or materials necessitating further testing with fish or higher vertebrates. This eliminates many compounds from further testing and reduces laboratory animal use.
- Use of cell culture or molecular biology in preliminary studies of basic mechanisms of cardiovascular disease. An example is the use of an immortal cell line in molecular research on the effects of oxygen on the chemotactic response of macrophages to oxygen, reducing the need for whole animal studies.



- \* Development of fish (rainbow trout, zebra danjo & medaka) as predictive models for epigenetic carcinogens has reduced mammalian animal use in carcinogenesis studies.
- \* Development and validation of fish immune responses as a biomarker to replace laboratory mammals.
- \* Purchase of elutriation system reduced the number of mice required for Modulation of Kupffer Cell Tumoricidal Properties by 50%.
- \* Toxin and toxoid preparation are titrated in a newly developed cell assay to minimize the use of animals for dose determination.
- Development of an *in vitro* test for cytoadherence by malaria-infected erythrocytes to human melanoma cells, umbilical vein cells, and endothelial cells greatly reduces the need for nonhuman primates.
- Development of a severe combined immunodeficiency disease mouse model where transplanted human liver tissue, a target for malarial sporozoite infection, cannot be rejected, permits the evaluation of potential malarial vaccine candidates in a non-monkey model.
- Development of an *in vitro* drug screening system using infected human cells to replace the mouse malaria lethality model, eliminating the need for 4,000 mice per year.
- *In vitro* drug screening, drug release kinetics, etc., result in reduction of drug candidates for numerous toxins reducing *in vivo* testing in rodent models up to 90% in some studies.
- Significant effort to develop DNA probes to detect *Rickettsia tsutsugamushi* in mammalian (including human) and chigger tissues should result in a 50% decrease in animal use for isolation and detection of this infectious agent.
- Development of an *in vitro* cultured human hepatoma cell line to assess radical and curative prophylactic activity of antimalarial drugs is in progress. This has the potential to reduce the number of monkeys needed for assessing antimalarial drugs and related compounds.
- *In vitro* techniques using human bone marrow cell culture to demonstrate propagation of Dengue viruses in these cells have reduced the number of monkeys needed for viral propagation by 25%.
- Development of a mosquito model using *in vitro* Dengue antigen detection techniques to pre-screen Dengue candidate vaccines should reduce the number of nonhuman primates needed for evaluation of vaccine candidates.

- Development of a reliable cell culture system for evaluating *Rickettsia tsutsugamushi* antibiotic resistance has reduced the need for animals for drug resistance studies by 50%.
- DNA probes have been developed to screen human *E. coli* isolates for pathogenicity. Only those positive to *in vitro* screening are tested in animals to confirm pathogenicity; this greatly decreases the numbers of animals used.
- Use of ELISA (enzyme linked immunosorbent assay) tests as a first screen in cellular mediator (interleukin 1) studies has reduced the number of mice previously required by 90%.
- The nervous systems of invertebrate sea slugs are used to study the effect of chemical and toxic agents on the electrical properties of nerve cells. This preliminary work reduces the number of vertebrates needed for subsequent study.
- Development and use of amphibian models (*Xenopus laevis* - frog) for assessing teratogenesis assays significantly reduce mammalian animal use.
- \* Interlaboratory validation of the Frog Embryo Teratogenesis Assay in collaboration with NTP-NIEHS. On-going work with NTP-NIEHS to develop non-mammalian alternative methods for neurobehavioral and reproductive toxicology endpoint assessments. Collaborative work with NIEHS to use genetically engineered fish to investigate the effects of environmental contamination.

#### VI.2.2.B Reduction by Substitution of *in vitro* or *ex vivo* Methods

- Synthetic *in vitro* or *ex vivo* systems like artificial bimembrane layers, cell or tissue culture systems, and isolated diaphragm muscle preparations replace or reduce the need for live, whole animal experiments in medical chemical defense research.
- Perfection of an *in vitro* method for growing *Plasmodium falciparum* (the most important human malaria that affects only man and certain monkey species) in human red blood cells has greatly reduced the number of nonhuman primates needed for this research.
- Development of specialized insect and vertebrate cell lines have reduced the need for intracerebral inoculation of suckling mice for the isolation of arboviruses.
- Use of transformed (immortal or self-propagating) cell cultures as an alternative to primary cell cultures that require frequent harvesting of tissues from animals.

- The use of monoclonal antibodies from hybridoma cells to replace animal-derived polyclonal antibody preparations greatly reduces animal requirements.
- \* Tissue culture of mouse osseous cells used as a reduction strategy for live animals to study biocompatibility of dental impression materials.
- *In vitro* techniques to orally infect mosquitoes with Dengue viruses have reduced the number of mice and monkeys needed for viral propagation by 25%.
- \* Development of new technology utilizing tissue slices from dead animals to assess the toxicity of selected environmental contaminants.
- \* Use of isolated perfused liver preparation to study the hepatotoxic effects of selected chemicals.
- \* Use of cultured cells for cytochrome P450 induction in vertebrate endothelium. Cells from 6 pigs represented the equivalent of approximately 100 pigs for *in vivo* studies.
- \* Cell cultures being developed to study mechanism of cyclic hydrocarbons and heavy metal toxicity.

#### **VI.2.2.C Reduction by Substitution of Another Animal Species, or Human Subjects as Protocols Progress into Human Trials**

- Studies have been performed to develop mouse and guinea pig models to replace the monkey as an aerosol model for botulism, staphylococcal enterotoxin B, and plague intoxication, which greatly reduces the number of monkeys needed for biological product toxicity and protective efficacy testing.
- Progression of a model of anti-malaria protective immunity into humans, where protective immunity is induced in human subjects by injected irradiated malarial sporozoites, has reduced the need for animal use in malaria research.
- Although cynomolgus monkeys are the only known model for Hepatitis E infection, rats, lesser bandicoots (rat-like animal) and swine are being evaluated as alternate models to reduce the need for monkeys.

#### **VI.2.2.D Reduction by Substitution of Computer Simulations or Other Technologies**

- Use of bioengineering tools to measure physiological parameters on human subjects in operational and experimental gravity tolerance environments

may result in a decrease in the number of animals currently used in gravity tolerance work.

- A research effort is aimed at developing physiologically based computer models/algorithms to predict *in vivo* distribution, uptake, and elimination of toxic chemicals, thus reducing the need for animals.
- Development of a computer model simulating *in vivo* absorption, distribution, metabolism, and toxic effects of nerve agents and vesicants and validated against *in vivo* pharmacokinetics data in guinea pigs for the nerve-agent soman will significantly reduce the number of animals used in nerve-agent research.
- Training of professionals by interactive videos and innovative teaching techniques, e.g., laparoscopic instruments on synthetic sponges, reduces the use of animals.
- Integration of mathematical modeling and aeromedical cardiovascular nonhuman primate research should reduce animal use.
- A computer modeling program reduces the use of sheep in blast overpressure research.
- A computer modeling program that identifies active sites on large molecular weight toxin molecules for intervention with therapeutic drugs is underway. This effort will substantially reduce the numbers of animals used in biotoxin studies.
- \* Development of a model to understand the propagation and bioeffects of electromagnetic energy should reduce the number of animals used.
- \* Physiologically Based Pharmacokinetic Modeling to predict toxicity and metabolism of trichloroethylene, vinyl chloride and their mixtures, by oral and inhalation routes reduces the use of mice and rats.
- \* Development of a computer model to predict the distribution and toxic effect of candidate replacement fire extinguishing agents. This technique will reduce the use of rats.

#### **VI.2.2.E Reduction by Sharing Animals between Research Investigations**

- \* Use of the same control animals for more than one protocol reduces the number of animals required.

- \* By combining anesthesia and surgical demonstrations in goats, the numbers were reduced from eight to four.
- \* Military working dogs scheduled for euthanasia are used for training labs, while under anesthesia.
- \* Guinea pig tissue, required for an improved histology method for hydration and preservation of tissue morphology, is taken from guinea pigs used in other projects. Since animals are used twice, it reduces the total number of guinea pigs used per year.
- \* The effect of magnesium on ventricular rate control during a trial fibrillation was studied using pigs transferred from another protocol. The re-use of swine reduced the total number of swine used per year.
- \* Temperature monitoring during craniotomy procedures was carried out in conjunction with another protocol requiring swine. Re-use of swine reduced the total number of swine used per year.
- \* Training in trans-septal right heart catheterization utilized sheep being euthanized as part of another protocol. Re-use of sheep reduced the total number of sheep.
- \* Hearts from rats used in other experiments were utilized in studies of Growth and Characterization of Rat Cardiac Myocytes in a Capillary Cell Culture System - on Earth and in Space.
- \* Gastrointestinal tracts from baboons used in experiments at an independent research foundation were obtained and used in Postnatal Gastrointestinal Adaptation in Extremely Preterm Baboons with Respiratory Insufficiency: Effects of Trophic Feeds.

### **VI.2.3 Refinement**

The refinement alternative for animal use addresses the need to ensure that the maximum humane use of each animal is obtained through proper protocol design and efficient utilization of animals, or through the modification of the experimental design to reduce the ethical cost associated with the study.

#### **VI.2.3.A Refinement to Protocols that Reduce Pain**

- *Ex vivo* cardiovascular response studies (using tissues in isolated systems) of toxins eliminate potential pain and distress for animals that would be used in whole animal systems.

- Refinement of methodologies associated with the feeding of arthropod vectors (chiggers) on rodents reduces discomfort to the animals. Use of an unobtrusive barrier system to prevent escape of the chiggers eliminates the need for the attachment of a cumbersome feeding capsule on the anesthetized animal.
- Studies performed to compare less reactogenic adjuvant regimens and alternative sites to foot pad injections in guinea pigs for evaluating hypersensitivity reactions (inflammation and swelling) from candidate Q-fever vaccines decrease potential discomfort associated with evaluation of vaccine candidates.
- Sophisticated technology such as Nuclear Magnetic Resonance Imaging is used to follow biochemical changes occurring over time in rats and other animals. This non-invasive procedure results in the use of far fewer animals and a more physiologically normal model.
- Development and evaluation of micro-encapsulated, time-released anesthetics and analgesics potentially beneficial to casualties on the battlefield have been performed. If perfected, these compounds will provide long-acting analgesia or anesthesia for animals on research projects where anesthesia or analgesia is not currently feasible.
- An evaluation of the feasibility and effectiveness of using topical analgesia (pain relief) on rabbits in Draize eye irritancy testing, and in systemic analgesia during Sereny' Testing (inflammation bioassay) on guinea pigs was performed. This provides the ability to perform a test while decreasing pain and distress without altering the outcome.
- A transdermal (applied to the skin) delivery system of analgesia to relieve pain in dogs was evaluated. Provides an extended analgesia or anesthesia for animals on research projects, and will be of benefit in human and veterinary medicine for the relief of pain.
- \* Use of long-acting local anesthetic in addition to general anesthesia and post-op analgesics to relieve pain in graft adhesion studies in rabbits, pericranium tissue barrier in mandibular reconstruction studies in sheep, and pleurodesis by thoracoscopic microfibrillar collagen studies in the pig. A specially designed sling was used in the pig studies.
- \* In rabbit studies of repair of abdominal rectus fascia, long-acting post-op analgesics are used to reduce or eliminate pain.

### **VI.2.3.B Refinement to Protocols that Reduce Distress**

- Development of telemetric surgical procedures for implantation of sensors, allows non-stressful measurement of clinically relevant physiological parameters in non-clinical vaccine and drug efficacy studies. This not only decreases stress associated with manipulative measurements, but the radio-transmitted measurements vastly improve the quality and quantity of data available. Additionally, use of the telemetry allows physiological assessment for efficacy trials, makes intervention with analgesia more feasible, and significantly reduces the use of lethality as the primary endpoint.
- Video tapes are used for adjunct training of technicians and investigators for common animal use procedures, i.e., venipuncture, handling, and restraint.
- Novel antibody production and collection techniques in rabbits and goats with plasma collection chambers reduce potential distress associated with venipuncture procedures and reduce, and, in some cases, eliminate immunoadjuvant use.
- Use of slings for studies requiring restraint of pigs and extensive conditioning of the swine prior to initiation of the study result in a significant refinement by reducing potential distress.
- DoD facilities use social housing systems, e.g., multiple animal housing or gang caging, where feasible, which expand intraspecies interactions, and use environmental enrichment strategies that extend to many species that are not specifically mandated by animal welfare legislation. These housing strategies increase the quality of life for the animals.
- A flexible polyethylene mesh restraint device that is more comfortable and is well tolerated by rodents replaces the use of rigid restrainers previously used for maintenance of arthropod (mosquito) vectors.
- A project is underway that plays back natural nonhuman primate vocalizations and analyzes the effectiveness of this as an environmental enrichment strategy.
- Development of a hyphema (fluid in the anterior chamber) model in rabbits has been using a non-invasive laser beam to open intraocular vessels and to create the hyphema instead of the standard surgical procedure previously required. This procedure eliminates post-surgical distress.
- Study endpoints are adjusted to reduce the need to proceed to death as a defined protocol objective. An example is the evaluation of the neurotoxicity of candidate therapeutic radioprotective compounds in mice using decrements or changes in motor behavior and coordination as a definitive endpoint rather than death. Another example is using respiratory distress,

rather than death, as an endpoint in the *In vivo* Study of Enhancement of Cis-Platinum Antitumor Activity by Pentoxifyllin in Nude Mice with Human Ovarian Carcinoma.

- A non-lethal model of botulism that detects intoxication by sciatic nerve paralysis in mice is under development and will be a significant refinement to the current mouse bioassay.
- \* By increasing quarantine time by at least a week for goats used for training, stress-related illness and deaths were decreased.
- \* By creating a carotid loop, the hemodynamics of simulated amniotic fluid embolism could be studied on unanesthetized sheep with minimal restraint.
- \* Comparison of metabolic constants for halocarbons derived from animal studies can be used to enhance the predictive value of human *in vitro* data in the risk assessment process.
- \* Comparison of *in vitro* results using tissues derived from the same animal to help validate the *in vitro* assay as an alternative to live animal use in toxicology research.

#### VI.2.3.C Refinement in Research Models and Animal Alternatives

- Professional biostatisticians are used by IACUCs to collaborate with scientists on experimental design and to review proposals in committee to ensure that only the minimal numbers of animals needed for statistical validity are approved for use.
- Extensive use of purpose-bred, (e.g., nude mice, hairless guinea pigs) microbiologically and genetically defined research animals yields better animal models and more meaningful and relevant research results.

### VI.3 SUMMARY

Each year new techniques and capabilities improve the handling, treatment, and use of animals in research and testing, and potentially reduce the need for animals in those same endeavors. In FY94, there was ample evidence of the DoD's aggressive pursuit of alternatives to replace, reduce and refine the use of animals. The development of new alternatives was promoted by the DoD's investment of approximately \$2M in FY94. For example, these funds supported USAMRMC's STO on reducing reliance on animals for research and improving experimental conditions using animals, and the development of the Frog Embryo Teratogenesis Assay toxicity test. In addition to these developmental efforts, animal use data for FY94 indicate the widespread implementation of validated alternatives. As described in Section V, large numbers of fish were used in toxicity testing to replace



the use of mammals. Alternatives implementation manifested by fish usage accounted for over 18% of total animal use. Rats and mice continue to replace nonhuman primates and other mammals higher on the phylogenetic scale in vaccine and drug development efforts. These and other examples of the development and implementation of alternatives have translated into lower overall spending and reductions in the overall use of higher animals (see Section V). Animal use alternatives including refinement, reduction, and replacement constitute key initiatives in the biomedical research, testing, education, and training programs of the Department of Defense. The number of large animals used by the military departments over the past decade has been very significantly reduced, and some large species are rarely used at all. Dogs, cats, nonhuman primates, and marine mammals collectively now represent less than .6 % of the total animals used in research by the DoD.

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## GLOSSARY

## Section VII

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**Adjuvant:** An agent mixed in a vaccine to enhance the immunological protection afforded.

**Alternatives to Animal Use:** For purposes of this assessment, "alternatives" are defined as encompassing any subjects, protocols, or technologies that replace the use of laboratory animals altogether; reduce the number of animals required; or refine existing procedures or techniques so as to minimize the level of stress endured by the animal. These technologies involve the continued, but modified, use of animals; use of living systems; use of chemical and physical systems; and use of computers.

**American Association for Accreditation of Laboratory Animal Care (AAALAC):** A voluntary private organization that, by Fall 1994, provided accreditation for 583 institutions. AAALAC accreditation is based on the provisions of the NIH *Guide for the Care and Use of Laboratory Animals*, and is recognized by the Public Health Service.

**Analgesic:** An agent that relieves pain without causing loss of consciousness.

**Anesthetic:** An agent that causes loss of the sensation of pain. Anesthetics may be classified as topical, local, or general.

**Animal:** For purposes of this assessment excluding embryos, animal is defined as any nonhuman member of five classes of vertebrates: mammals, birds, reptiles, amphibians, and fish. Within this group, two kinds of animals can be distinguished, warm-blooded animals (mammals and birds) and cold-blooded animals (reptiles, amphibians, and fish). Under this definition, invertebrates are not included.

**Animal Care and Use Committee (ACUC):** See Institutional Animal Care and Use Committee (IACUC).

**Animal Use:** The use of animals for research purposes. Three aspects of animal use are dealt with in this assessment: in behavioral and biomedical research; in testing products for toxicity; and in the education of students at all levels. This assessment does not cover animal use for food and fiber; animal use to obtain biological products; or animal use for sport, entertainment, or companionship.

**Animal Welfare Act:** This act, passed in 1966 and amended in 1970, 1976, and 1985, was originally an endeavor to stop traffic in stolen animals that were being shipped

across State lines and sold to research laboratories. Amendments to the act have expanded its scope to include housing, feeding, transportation, and other aspects of animal care; however, the act bars regulation of the conduct of research and testing by USDA. Animals covered by the act, as currently enforced, are dogs, cats, hamsters, rabbits, guinea pigs, nonhuman primates, and marine mammals.

**Antibody:** Proactive proteins produced by lymphocytes (type of white blood cell) that can specifically bind foreign substances.

**Biological Model:** A surrogate or substitute for a process or organ of interest to an investigator. Animals or alternatives can serve as biological models.

**Biological Testing:** The repetitive use of a standard biological test situation or protocol employing different chemicals or different test parameters. Such test protocols are more stereotyped than those used in research, and may be more amenable to the institution of a computerized data retrieval system.

**Biomedical Research:** A branch of research devoted to the understanding of life processes and the application of this knowledge to serve humans and animals. A major user of animals, biomedical research affects human health and the health care industry. It is instrumental in the development of medical products such as drugs and medical devices, and in the development of services such as surgical and diagnostic techniques. Biomedical research covers a broad spectrum of disciplines, such as anatomy, biochemistry, biology, endocrinology, genetics, immunology, nutrition, oncology, and toxicology.

**Blast Overpressure:** The concussion that results when weapons such as artillery pieces are fired. Soldiers firing these weapons can be severely injured by the local pressure effects resulting from weapon use. Blast overpressure occurs when soldiers are fired upon also - i.e., the shock wave from enemy weapon fire/blast.

**Carcinogen:** An agent or process that significantly increases the incidence of abnormal, invasive, or uncontrolled cell growth in a population. Carcinogens fall into three classes: chemicals, viruses, and ionizing radiation. A variety of screening assays have been developed to detect chemical carcinogens, including the *Salmonella*-mediated mutagenesis assay (Ames test), the sister chromatid exchange assay, and traditional laboratory animal toxicity tests.

**Carcinogenesis:** The process by which a change to a cell occurs that leads to cancer.

**Cell Culture:** Growth in the laboratory of cells isolated from multicellular organisms. Each culture is usually of one type. Cell culture may provide a promising alternative to animal experimentation, for example, in the testing of mutagenicity, and may also become a useful adjunct in repeated-dose toxicity testing.

**Chemotactic:** To attract by release of a chemical. For example, cells are attracted to a site of tissue damage by the release of chemicals by the injured cells.

**Computer Simulations:** The use of specially devised computer programs to simulate cells, tissues, fluids, organs, and organ systems for research purposes: to develop mathematical models and algorithms for use in toxicity testing, and to simulate experiments traditionally done with animals for educational purposes.

**Distress:** Usually the production of pain, anxiety, or fear. However, distress can also occur in the absence of pain. For example, an animal struggling in a restraint device may be free from pain, but may be in distress. Distress can be eased with tranquilizers.

**Draize Eye Irritancy Test:** A test that involves placing a single dose of a test substance into one eye of four to six rabbits (the other eye remains untreated) and observing its irritating effects. A promising alternative to this test is the chick embryo chorioallantoic membrane assay.

**Education:** The aspect of education dealt with in this assessment is the use of animals and alternatives in the teaching of life sciences to health professionals and preprofessionals, and research scientists.

**ELISA (Enzyme Linked Immunosorbent Assay):** An assay system that uses antibodies conjugated to enzymes. The amount of antibody attached to the molecule being analyzed can be detected by adding compounds that are cut by the enzyme releasing a colored product which can be quantified.

**Ex vivo:** Outside the living body: denoting removal of an organ, tissue or cells.

**Guidelines for Animal Care and Use:** Various organizations outside the Federal Government have adopted their own guidelines -- e.g., the American Psychological Association's *Guidelines for Ethical Conduct in the Care and Use of Animals*, which is comprehensive and has been endorsed by FASEB; the American Physiological Society's *Guiding Principles in the Care and Use of Animals*; and the American Veterinary Medical Association's *Animal Welfare Guiding Principles*. For federal guidelines, see Interagency Research Animal Committee, NIH *Guide for the Care and Use of Laboratory Animals*, and PHS Policy.

**Institute of Laboratory Animal Resources (ILAR):** A component of the National Research Council, ILAR performs periodic surveys on the use of laboratory animals.

**Institutional Animal Care and Use Committee (IACUC):** An institutional committee that reviews research proposals and oversees housing and routine care of animals. The committee's membership generally includes the institution's attending veterinarian, a representative of the institution's administration, users of research animals, and one or more nonscientist and lay member.

**Invertebrate:** Any nonplant organism without a spinal column -- e.g., worms, insects, and crustaceans. Invertebrates account for 90 percent of the Earth's nonplant species. For the purposes of this assessment, invertebrates are not considered to be animals.

**In vitro:** Literally, in glass; pertaining to a biological process or reaction taking place in an artificial environment, usually a laboratory. Human and animal cells, tissues, and organs can be cultured *in vitro*. *In vitro* testing may hold some promising alternatives to animal testing -- e.g., in testing for eye irritation and mutagenicity.

**In vivo:** Literally, in the living; pertaining to a biological process or reaction taking place in a living cell or organism.

**Macrophage:** A white blood cell that is very active in inflammatory responses and in engulfing foreign objects such as bacteria.

**Mutagenesis:** An agent that induces chemical changes in genetic material. Chemicals, viruses, and ionizing radiation can be mutagenic. Most carcinogens are mutagens; therefore, many screening tests to detect carcinogens are designed to detect the mutagenic potential of the compound. Some mutagens are not direct-acting, requiring metabolic activation in the body before they exert their mutagenic potential.

**National Institutes of Health's *Guide for the Care and Use of Laboratory Animals*:** Revised in 1985, the *Guide* lays out detailed standards for animal care, maintenance, and housing. Its provisions apply to all research supported by NIH, and it is used by many animal research facilities, both within and outside the Federal Government. AAALAC and PHS also use it when assessing research facilities for accreditation.

**Organ Culture:** The attempt to isolate and maintain animal or human organs in *in-vitro* culture. Long-term culture of whole organs is not generally feasible, but they can be sustained in cultures for short periods (hours or days).

**Pain:** Discomfort resulting from injury or disease. Pain can also be psychosomatic, the product of emotional stress. Pain can be induced by mechanical, thermal, electrical, or chemical stimuli, and it can be relieved by analgesics or anesthetics.

**Public Health Service *Policy on Humane Care and Use of Laboratory Animals*** Revised in 1985, the *Policy* applies to PHS-supported activities involving animals (including those of NIH). It relies on the NIH *Guide for the Care and Use of Laboratory Animals*, and uses institutional committees for the assessment of programs and maintenance of records.

**Polymerase Chain Reaction:** A molecular biological system in which pieces of genetic material can be synthesized in large amounts *in vitro*. This material can be used in diagnostic testing, genetic studies, or for a large number of molecular biological purposes.

**Protocol:** The written plan of a scientific experiment or treatment.

**Reduction:** Considered an alternative to animal use when fewer animals are used in research and education through changed practices, sharing of animals, or better design of experimental protocols.

**Refinement:** An alternative to animal use by better use and modification of existing procedures so that animals are subject to less pain and distress. Examples of such refinements are the administration of anesthetics and tranquilizers, humane destruction, and the use of noninvasive imaging techniques.

**Replacement:** An alternative to animal use, replacing methods using animals with those that do not. Examples include the use of a placenta instead of a whole animal for microsurgical training, the use of cell cultures instead of mice and rats, the use of non-living systems, and the use of computer programs.

**Research Facility:** Under the Animal Welfare Act, any individual, institution, organization, or postsecondary school that uses or intends to use live animals in research, tests, or experiments. Facilities that receive no federal support for experimental work and that either purchase animals only within their own state or that maintain their own breeding colonies are not considered research facilities under the act, however.

**Sporozoite:** The infectious stage of the malarial parasite that is transmitted by mosquitoes.

**Testing:** Standardized procedures that have been demonstrated to predict certain health effects in humans and animals. Testing involves the frequent repetition of well-defined procedures with measurement of standardized biological endpoints. A given test may be used to evaluate many different substances and use many animals. Testing is used to establish the efficacy, safety, and toxicity of substances and procedures.

**Tissue Culture:** The maintenance *in vitro* of isolated pieces of a living organism. The various cell types are still arranged as they were in the original organism and their differential functions are intact.

**Toxicity Testing:** The testing of substances for toxicity in order to establish conditions for their safe use. There are now more than 50,000 chemicals on the market and 500 to 1,000 new ones are introduced each year.

**Vesicant:** A chemical agent that causes burns and tissue destruction both internally and externally.

**Veterinary Medicine:** The science and art of prevention, cure and/or alleviation of disease and injury in animals. Veterinary medicine includes the management of animal care and use programs.

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## Section VIII

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